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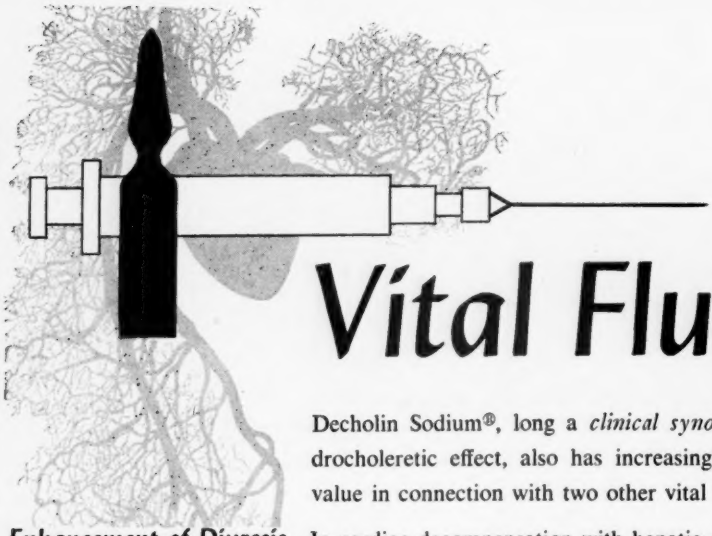

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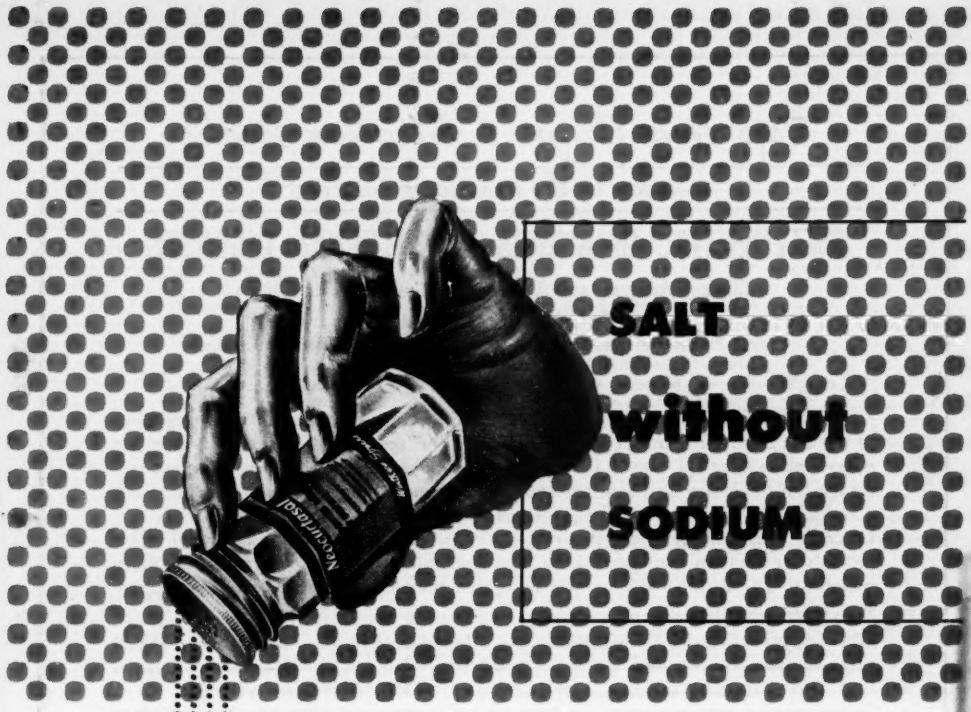

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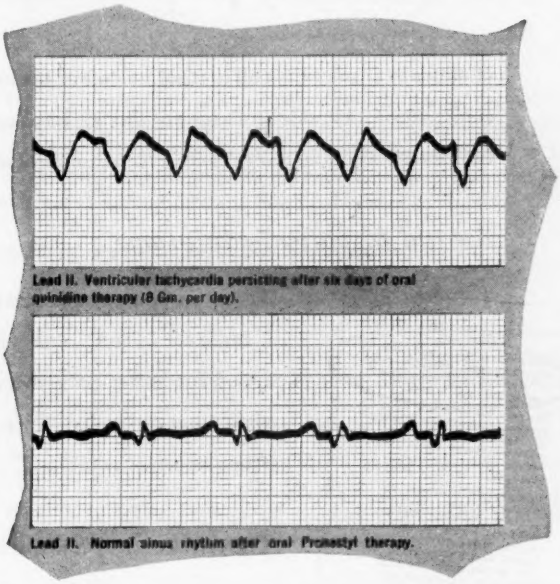

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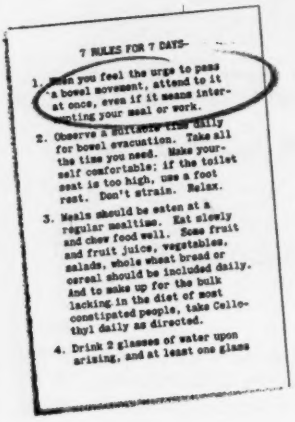
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1. *Bargen, J. A.: Gastroenterology 13:275, 1949.*
2. *Musick, V. H.: J. Oklahoma M. A. 43:360, 1950.*
3. *Schweig, K.: New York State J. Med. 48:1822, 1948.*
4. *Council on Pharmacy and Chemistry: J.A.M.A. 143:897, 1950.*

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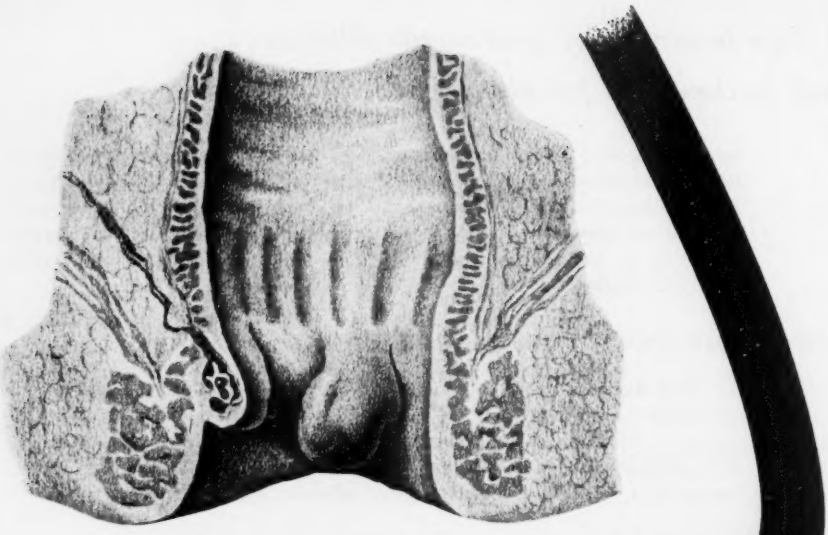

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ARCHIVES of INTERNAL MEDICINE

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EFFECTS OF TERRAMYCIN ON THE BACTERIAL FLORA OF THE BOWEL IN MAN

JOSEPH M. DI CAPRIO, M.D.
AND
LOWELL A. RANTZ, M.D.
SAN FRANCISCO

THE PRESENT study was primarily undertaken to determine the action of terramycin [1] on the bacterial flora of the bowel in man, particularly in the presence of ulcerative lesions of that organ.

METHODS

Stool specimens were collected in clean, wax-coated cardboard containers and cultured the same day. They were obtained before and within 36 hours after the institution of therapy in every instance, and at varying intervals thereafter depending on the nature of the case. A total of 44 stool specimens was examined.

There were two objectives in the cultural study: to make a quantitative, as well as qualitative, determination of the micro-organisms present in the stool, and to determine the in vitro sensitivity to penicillin, dihydrostreptomycin and terramycin of the micro-organisms identified. The procedure was as follows: One milliliter of wet stool was added to 9 ml. of sterile isotonic sodium chloride solution and stirred to make a 1:10 dilution. Serial dilutions of 1:100, 1:1,000 and 1:10,000, also in isotonic sodium chloride solution, were made from this. Only two of the above dilutions were used in the culturing of any given specimen. As a rule, for the original specimen the 1:100 and 1:10,000 dilutions were employed and for the second the 1:10 and 1:1,000 dilutions.

The following mediums, contained in Petri dishes in solid form, were used for each specimen routinely: plain blood agar; MacConkey's medium; blood agar containing 0.1 unit of penicillin per milliliter of medium; blood agar containing 0.4 unit of penicillin per milliliter of medium; blood agar containing 10 micrograms of dihydrostreptomycin per milliliter of medium; blood agar containing 5 micrograms of terramycin hydrochloride per milliliter of medium.

A 0.1 ml. volume of each dilution chosen was inoculated on each of the six different mediums. Since two dilutions were used in every instance, a total of 12

From the Department of Medicine, Stanford University School of Medicine.

Miss Gail Reeves and Miss Anne Zuckerman assisted in this study. Drs. Emile F. Holman, Robert A. Scarborough, Russell R. Klein, Clyde C. Greene Jr. and Claude P. Callaway made available some of the patients included in this study.

1. Two forms of terramycin were used in this study, amphoteric terramycin and the salt, terramycin hydrochloride. Both forms of the drug were obtained from Chas. Pfizer & Company, Inc., Brooklyn, through the courtesy of Dr. Gladys Hobby.

plates was employed in the culturing of each specimen. All plates were incubated aerobically at 37 C. (98.6 F.) and read the following day. No anaerobic cultures were made. Isolated organisms were identified by appropriate microscopic and biochemical methods.

A total of seven patients was included in this study. The dosage commonly employed was 750 mg. of terramycin hydrochloride given orally every six hours, or four times a day, for a daily total of 3 Gm. Two of the patients received 500 mg. of amphoteric terramycin, a less soluble form than the salt, terramycin hydrochloride, and presumably less well absorbed, orally every six hours for a daily total of 2 Gm. The duration of therapy varied from two to 22 days, depending in large measure on the nature of the case.

CLINICAL NOTES

PATIENT 1.—Diagnosis: Regional ileitis. A 26 year old man had a sudden onset of diarrhea and abdominal cramps two years ago. A diagnosis of regional ileitis was made, and nine months ago an ileocolostomy was performed, with exclusion of the diseased portion of the ileum. His condition improved temporarily, but his symptoms returned and were persistent for five to six months.

The patient received 750 mg. of terramycin hydrochloride four times a day orally for 12 days. While receiving the drug, and for one week following discontinuance of its administration, the patient's condition did not improve. Subsequently, there was remarkable improvement, and, for the past two months (duration of follow-up period at time of present writing), except for a transient period of diarrhea lasting two to three days, he has had only one formed bowel movement daily.

PATIENT 2.—Diagnosis: Amebiasis. A 45 year old man had a history of bloody diarrhea and abdominal cramps of four weeks' duration. Cysts of Endamoeba histolytica were recovered from the stools. A barium sulfate enema revealed deformity of the cecum. Before therapy was begun diarrhea had ceased, but the patient still felt weak and ate poorly.

The patient received terramycin hydrochloride, 750 mg. orally four times a day for 22 days. After one week of therapy, the patient felt better. A stool was examined for E. histolytica after 13 days of therapy and found to be negative, as were three subsequent stools examined. The patient felt completely well at the completion of therapy. A repeat barium sulfate enema given three days after discontinuation of treatment with the drug showed only slight improvement in the cecal lesion.

PATIENT 3.—Diagnosis: Recurrent regional ileitis. The disorder of a 47 year old woman was diagnosed on a previous admission to the hospital 1½ years ago as regional ileitis. At that time a resection of the terminal portion of the ileum and ascending colon was performed, with an end to end ileocolostomy. The immediate postoperative course had been good, but, in the past several months, she had had a recurrence of diarrhea and abdominal cramps. A series of roentgenograms of the gastrointestinal tract revealed the presence of disease proximal to the anastomosis.

The patient received 500 mg. of amphoteric terramycin orally every six hours for a few days and then was given terramycin hydrochloride, 500 mg. orally every six hours. The total duration of terramycin therapy was 14 days. The patient's condition showed no significant improvement.

PATIENT 4.—Diagnosis: Rectovaginal fistula. A 30 year old woman first noted a discharge of pus from the rectum one year before her admission to the hospital.

The discharge ceased after the administration of aureomycin and chloramphenicol (chloromycetin®). Two months before her admission she again noted a discharge of pus from the rectum and from the vagina as well. She was placed on a daily dose of 2 Gm. of chloramphenicol, which she continued to take until admission. For the three weeks immediately prior to admission, the patient was free of symptoms.

The patient received terramycin hydrochloride, 750 mg. four times a day orally for two days. It was not possible to evaluate the efficacy of terramycin in this patient, since she was admitted for the express purpose of having a plastic repair of her rectovaginal fistula and received terramycin for only two days preoperatively.

PATIENT 5.—Diagnosis: Nonspecific ulcerative colitis. A 29 year old woman was first admitted to the hospital five months previously, at which time a diagnosis of nonspecific ulcerative colitis was made. An ileosigmoidostomy, together with an ileostomy and a sigmoidostomy, was performed. Immediately after the operation, the patient's condition improved dramatically. A few months later diarrhea and abdominal cramps developed, and she passed blood per rectum. Proctoscopy revealed a diffusely injected mucosa with superficial ulcerations. A barium sulfate enema showed recurrence of disease in the ileum and sigmoid colon in the region of the anastomosis.

Treatment was started with amphoteric terramycin, and, after a few days, terramycin hydrochloride was substituted, in the same dosage schedule of 500 mg. orally every six hours. She received terramycin in one form or the other for 10 days. The patient's condition showed no definite improvement.

PATIENT 6.—Diagnosis: Rectal ulcers and possible amebiasis. A 38 year old man had a duodenal ulcer demonstrated by roentgenogram one year previously which responded to a medical regimen. The present difficulty consisted of bloody diarrhea and abdominal cramps for 10 days. Proctoscopic examination revealed several ulcerations of the rectal mucosa, from one of which a motile ameba was recovered that may have been E. histolytica.

The patient received terramycin hydrochloride, 750 mg. orally four times a day for six days. He became asymptomatic after three days of therapy and remained so. Proctoscopic examination on the sixth day of therapy showed complete healing of the ulcers.

PATIENT 7.—Diagnosis: Herpes zoster. A 74 year old woman had typical herpes zoster. She was placed initially on a dosage schedule of 750 mg. of terramycin hydrochloride given orally every six hours. It was necessary to reduce the dosage to 250 mg. every six hours after three days because of the development of distressing gastrointestinal symptoms. She continued on the reduced dosage schedule for three more days. The course of her herpes zoster did not appear to be influenced by the therapy.

TOXICITY

The occurrence of untoward side effects from the terramycin, illustrated in table 1, was of interest. Periodic determinations of the hemoglobin concentration, white blood cell counts and routine urinalyses were carried out in almost all the patients. As shown in table 1, there was a high incidence of untoward side effects related to the gastrointestinal system. These consisted of epigastric burning or heartburn, anorexia, nausea and vomiting. Though prominent in some instances, they did not interfere with therapy over a short period.

Patient 1, who received terramycin for 12 days, showed a trace of protein in his urine, with 1 to 4 red blood cells per high power field in a centrifuged specimen on the last day of therapy. Urine examined two days later was normal. It has been reported that the elimination of the normal bacterial fecal flora may result in a decrease in plasma prothrombin. The finding of a trace of proteinuria and an increase in red blood cells in this patient may have represented a small renal hemorrhage. The possibility of direct renal toxicity, however, cannot be excluded, although this has not been reported to date.

No toxic effect on the hemoglobin concentration or white blood cell count was observed. There were no instances of drug fever or skin eruptions.

TABLE 1.—*Occurrence of Untoward Side Effects from Terramycin Therapy*

Patient	Gastrointestinal Symptoms	Toxic Effects on Renal and Hemopoietic Systems
1	None	Trace of proteinuria, with increase in red blood cells in urine
2	Nausea	None
3	Epigastric burning; nausea; vomiting	None
4	Nausea	Not determined
5	Nausea; vomiting	None
6	None	None
7	Anorexia; heart burn; nausea; vomiting	Not determined

ANALYSIS OF BACTERIOLOGIC DATA

The results of the bacteriologic examination of the stools before terramycin therapy, and on one or more occasions after therapy, are shown in table 2. The organisms most commonly present before therapy were members of the coliform group (i. e., Escherichia, Aerobacter aerogenes, intermediate forms and paracolon bacilli), nonhemolytic streptococci and Streptococcus faecalis. The number of organisms present was quite variable, depending in some measure on the underlying disease process.

The in vitro sensitivity tests of the organisms isolated before treatment revealed that most were resistant to penicillin and sensitive to dihydrostreptomycin; Str. faecalis was a notable exception. All the organisms were sensitive to terramycin, with the exception of Proteus and the yeasts.

In five of the seven patients (patients 2, 3, 5, 6 and 7) within 48 hours of therapy, all the bacteria present before therapy were either entirely eliminated or reduced to a total concentration of less than 2,000 organisms per milliliter of wet stool. In the remaining two patients (patients 1 and 4) no stool specimens were obtained at the end of 48 hours of therapy. In patient 1 a specimen obtained after four days of

therapy showed the presence of only 100 Escherichia organisms per milliliter of wet stool. Patient 4 showed some decrease in the total bacterial count after 24 hours. No further observations were made, but, in view of the resistance of the Proteus organisms to terramycin in the concentration tested, i. e., 5 micrograms per milliliter of medium, it is possible that this organism would not have been eliminated.

The question of development of resistant organisms was of interest. From the above tables and the results of stool examinations not shown, it was apparent that, as the normal bacterial flora was eliminated, it was supplanted by yeasts and Staphylococcus pyogenes var. aureus, coagulase negative, both of which were resistant to the concentration of terramycin used for the in vitro tests, i. e., 5 micrograms per milliliter of medium. Two of the patients (patients 5 and 6) showed the presence of terramycin-resistant paracolon bacilli in their stools after eight and two days, respectively, of terramycin therapy. None of the above organisms could truly be said to have developed resistance, since they either were not encountered in the same patient before therapy or, when encountered, were resistant to terramycin on original isolation. It should be noted, however, that all the paracolon bacilli that were isolated before therapy were sensitive to terramycin.

There was possibly one instance of true development of resistance. Terramycin-resistant Escherichia organisms were recovered from patient 1 six days after the discontinuance of terramycin administration. No such organisms were encountered, however, while he was receiving the drug.

Within one to several days after the discontinuance of therapy, the normal flora returned. As shown in table 2, concomitant with the elimination of the normal bowel flora, the stools tended to become greenish and to lose their odor. Their usual characteristics returned within one to several days after the normal bowel flora had reestablished itself.

COMMENT

This study indicated that terramycin could be effectively employed to prepare patients for surgical measures on the bowel, since its administration in an oral dosage of 3 Gm. daily resulted in the virtual elimination of the normal aerobic flora within 48 hours. Studies carried out by Herrell and his co-workers [2] on the pharmacologic aspects of terramycin hydrochloride showed that the drug was excreted in high concentration in the feces, levels ranging from 320 to 2,506 micrograms per milliliter having been obtained in patients receiving 4 Gm. by

2. Herrell, W. E.; Heilman, F. R.; Wellman, W. E., and Bartholomew, L. G.: Terramycin: Some Pharmacologic and Clinical Observations, Proc. Staff Meet., Mayo Clin. **25**:183, 1950.

TABLE 2.—*Results of Bacteriologic Examination of Stools Before and After Terramycin Therapy*

Therapy	Gross Description of Stool	Organisms	Bacterial Concentration per Ml. of Wet Stool	In Vitro Sensitivity*			
				Penicillin		Dihydrostreptomycin	Terramycin
				0.1 Unit/Ml.	0.4 Unit/Ml.	10 Micrograms/Ml.	5 Micrograms/Ml.
Patient 1							
None	Yellow; formed; fecal odor	Escherichia; A. aerogenes	50,000,000	R	R	S	S
				R	R	X	S
		Str. faecalis	1,000,000	R	R	R	S
Terramycin hydrochloride, 750 mg. q.i.d. for 1 day	Yellow, brown; thick, pasty; faint fecal odor	Escherichia; A. aerogenes	5,000,000	R	R	S	S
				R	R	X	S
Terramycin hydrochloride, 750 mg. q.i.d. for 4 days	Brown, green; thick, pasty; no odor	Escherichia	100	R	R	S	S
Terramycin hydrochloride, 750 mg. q.i.d. for 8 days	Yellow, green; thick, pasty; no odor	Plates sterile					
Patient 2							
None	Brown; formed; fecal odor	Nonhemolytic streptococci	100,000	R	S	R	S
		Paracolon bacilli	100,000	R	R	S	S
		Staph. pyogenes var. albus, coagulase negative; yeasts	100,000	R	R	X	X
				R	R	R	R
Terramycin hydrochloride, 750 mg. q.i.d. for 2 days	Green, brown; soft, formed; odorless	Yeasts	40,000,000	R	R	R	R
Terramycin hydrochloride, 750 mg. q.i.d. for 13 days	Brown; formed; odorless	Staph. pyogenes var. aureus, coagulase negative	40,000,000	R	S	S	R
		Yeasts	10,000,000	R	R	R	R
Patient 3							
Previously received succinylsulfathiazole; 300,000 units procaine penicillin intramuscularly daily for 3 days; no terramycin	Brown; liquid; fecal odor	Paracolon; intermediate coliform unidentified	25,000,000	R	R	S	S
		gram-negative rod		R	R	S	S
		Str. faecalis	3,000,000	R	R	R	S
Amphoteric terramycin, 500 mg. q. 6 hr. for 5 doses (30 hr.); also 300,000 units procaine penicillin intramuscularly daily	Green, brown; liquid, with suspended solid particles; no odor	Intermediate coliform	2,000	R	R	S	S
		Yeasts	3,000,000	R	R	R	R
Terramycin, either amphoteric or hydrochloride, 500 mg. q. 6 hr. for 8 days; penicillin not given for 5 days	Green; liquid, with suspended particles; no odor	Staph. pyogenes var. aureus, coagulase negative; yeasts	3,000,000	R	R	R	R
				R	R	R	R

Patient 4							
Chloramphenicol, 500 mg. q.i.d. for previous 2 mo.	Brown; formed; fecal odor	Proteus	†	R	R	S	R
		Paracolon; Escherichia	80,000,000	X	X	R	S
				X	X	S	S
		Str. faecalis	100,000,000	X	X	R	S
Terramycin hydrochloride, 750 mg. q.i.d. for 1 day; chloramphenicol not given 1 day	Gray, brown; formed; fecal odor	Proteus	†	R	R	S	R
		Paracolon; Escherichia	50,000,000	X	X	R	S
				X	X	S	S
		Str. faecalis	30,000,000	X	X	R	S
Patient 5							
Succinylsulfathiazole, 2.5 Gm. 5 × daily for 2 days; no terramycin	Brown; mucoid; fecal odor	Escherichia	12,500,000	R	R	S	S
		Str. faecalis	80,000,000	R	R	R	S
Succinylsulfathiazole, 2.5 Gm. 5 × daily for 4 days; amphoteric terramycin, 500 mg. q. 6 hr. for 6 doses (36 hr.)	Green, yellow; mucoid; no odor	All plates sterile					
Terramycin, amphoteric or hydrochloride, 500 mg. q. 6 hr. for 8 days; succinylsulfathiazole not given 7 days	Green; semiformed, mucoid; no odor	Paracolon bacilli	40,000,000	R	R	R	R
		Yeasts	12,000,000	R	R	R	R
Patient 6							
None	Brown; pasty	Escherichia; unidentified gram-negative rod; nonhemolytic streptococci	Quantitative studies not made	R	R	S	S
				R	R	S	S
				S	S	R	S
Terramycin hydrochloride, 750 mg. q.i.d. for 1 day	Green; pasty	All plates sterile					
Terramycin hydrochloride, 750 mg. q.i.d. for 6 days	Green; soft, formed; odorless	Paracolon; yeasts	Quantitative studies not made	R	R	S	R
				R	R	R	R
Patient 7							
None	Brown; soft, formed; fecal odor	Escherichia; paracolon	70,000	R	R	R	S
				R	R	S	S
		Nonhemolytic streptococci	40,000	S	S	S	S
Terramycin hydrochloride, 750 mg. q. 6 hr. for 2 days	Green, brown; soft, formed; fecal odor	Nonhemolytic streptococci	2,000	S	S	S	S
		Staph. pyogenes var. aureus, coagulase negative; yeasts	75,000	R	R	R	R
				R	R	R	R
Terramycin hydrochloride, 750 mg. q. 6 hr. for 3 days	Green, brown; soft, formed; no odor	Staph. pyogenes var. aureus, coagulase negative; yeasts	2,000,000	R	R	R	R
				R	R	R	R

* R indicates resistant organisms, S, sensitive organisms and X, sensitivity not determined.
† Proteus was a spreader.

mouth daily. These same authors also reported that terramycin greatly altered the aerobic and anaerobic flora of the bowel.

Terramycin compared favorably with other antimicrobial agents which have been employed for the preparation of patients for surgical treatment of the bowel and the relative efficacy of which has been disputed.

Poth [3] reported that the oral administration of succinylsulfathiazole (sulfasuxidine®) or phthalylsulfathiazole (sulfathalidine®) caused a significant reduction of coliform and anaerobic organisms in the bowel in three to seven days in 95 per cent of cases. He recommended that these drugs be given for seven days preoperatively.

Bacon and Rowe [4] observed that streptomycin appeared more effective in reducing the coliform count than did phthalylsulfathiazole, and the action of both seemed better than that of one alone. Pulaski and Connell [5] also reported that streptomycin was more effective against the coliform flora than the sulfonamides, and that the use of such substances as aluminum pectinate and glucurono-lactone inhibited the emergence of streptomycin-resistant strains.

Lockwood and his associates [6] found results with streptomycin given orally inconsistent. In their opinion this drug should not be used in the preoperative preparation of a patient for surgical measures on the bowel. A combination of streptomycin and relatively small doses of phthalylsulfathiazole did not give results significantly different from those with streptomycin alone.

Strax and Wright [7] reported aureomycin to be an effective agent in the preparation of patients for surgical treatment of the bowel, as effective as the insoluble sulfonamides and more rapid in its action. Pulaski and Connell,[5] on the other hand, in their report on a small series of cases, expressed the view that aureomycin was less effective than the sulfonamides or streptomycin.

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3. Poth, E. J.: Sulfasuxidine and Sulfathalidine in Surgery of the Colon, South. M. J. **40**:369, 1947.

4. Bacon, H. E., and Rowe, R. J.: Surgery of the Lower Bowel: Preparation and After-Care of Patient, J. A. M. A. **136**:975 (April 10) 1948.

5. Pulaski, E. J., and Connell, J. F., Jr.: Control of Peritoneal Infection in Gastrointestinal Surgery, Bull. U. S. Army M. Dept. **9**:265, 1949.

6. Lockwood, J. S.; Young, A. D.; Bouchelle, M.; Bryant, T. R., Jr., and Stojowski, A. J.: Appraisal of Oral Streptomycin as an Intestinal Antiseptic, with Observations on Rapid Development of Resistance of E. Coli to Streptomycin, Ann. Surg. **129**:14, 1949.

7. Strax, S., and Wright, L. T.: Recent Advances in Antibiotic Therapy, New York State J. Med. **49**:1797, 1949.

Dearing and Heilman [8] carried out an extensive study regarding the relative effectiveness of aureomycin alone, a combination of aureomycin and dihydrostreptomycin, phthalylsulfathiazole alone, succinylsulfathiazole alone and dihydrostreptomycin alone in the preparation of patients for surgical therapy of the bowel. Aureomycin alone appeared superior to either the insoluble sulfonamides alone or dihydrostreptomycin alone. Combined with dihydrostreptomycin, aureomycin appeared to be slightly enhanced in effectiveness.

The results obtained with terramycin in this study compared favorably with those obtained by Dearing and Heilman [8] with the use of aureomycin alone or a combination of aureomycin and dihydrostreptomycin. Perhaps, if a larger number of patients had been studied, more persons harboring terramycin-resistant Proteus or Pseudomonas organisms might have been detected. This possibility raised the question of the advisability of using dihydrostreptomycin in conjunction with terramycin.

The high incidence of gastrointestinal symptoms encountered in this study which were attributable to terramycin deserves comment. The group may have been unusual in this regard in view of the fact that two of the patients (patients 3 and 5) who had previously received succinylsulfathiazole also experienced nausea and vomiting with that drug, with which the expected incidence of side reactions is 1 per cent.[3] Terramycin was less toxic than aureomycin according to Herrell and his collaborators.[2]

SUMMARY

Terramycin, both in the amphoteric form and as its salt, the hydrochloride, had a pronounced effect on the bacterial flora of the healthy and diseased bowel of man, which was evident after 36 to 48 hours of therapy on an oral dosage schedule of 3 Gm. daily. Its effectiveness as an antimicrobial agent in the preparation of patients for surgical measures on the bowel appeared unexcelled.

8. Dearing, W. H., and Heilman, F. R.: The Effect of Aureomycin on the Bacterial Flora of the Intestinal Tract of Man: A Contribution to Preoperative Preparation, Proc. Staff Meet., Mayo Clin. **25**:87, 1950.

CLINICAL, FUNCTIONAL AND NEEDLE BIOPSY STUDY OF THE LIVER IN ALCOHOLISM

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THE DEVELOPMENT and increased use of the needle biopsy technic for the purpose of obtaining specimens of the liver have widened the field for the study of hepatic disease.[1] Among the published investigations several are concerned with the hepatic changes observed in alcoholism.[2] The purpose of this communication is to present the clinical, functional and histologic observations on a group of alcoholic patients and to describe the histologic changes in the liver noted at short intervals in the absence of specific therapy. Patients with obviously far advanced hepatic disease were excluded in the hope that early or limited lesions might be observed and their possible significance evaluated.

From the First Medical Division and the Laboratory of Pathology, City Hospital, Welfare Island.

1. (*a*) Iverson, P., and Roholm, K.: On Aspiration Biopsy of the Liver, with Remarks on Its Diagnostic Significance, Acta med. Scandinav. **102**:1-16, 1939. (*b*) Gillman, T., and Gillman, J.: A Modified Liver Aspiration Biopsy Apparatus and Technique, with Special Reference to Its Clinical Application as Assessed by 500 Biopsies, South African J. M. Sc. **10**:53-66 (June) 1945. (*c*) Hoffbauer, F. W.: Needle Biopsy of the Liver, J. A. M. A. **134**:666-670 (June 21) 1947.

2. (*a*) Chalmers, T. C.; Murphy, T. L., and Taft, E. B.: Incidence, Character, and Course of Liver Disease in Chronic Alcoholics as Determined by Needle Biopsy, J. Clin. Investigation **27**:528-529 (July) 1948. (*b*) Sanes, S.; Bahn, R. C.; Chapple, W. H. C., and Chasin, N.: Needle Biopsy of the Liver in Alcoholic Patients, New York State J. Med. **49**:2677-2681 (Nov. 15) 1949. (*c*) Buck, R. E.: Observations on Fatty Alcoholic Liver: The Use of Interval Needle Biopsy and Liver Function Tests, J. Lab. & Clin. Med. **33**:555-564 (May) 1948. (*d*) Volwiler, W., and Jones, C. M.: The Diagnostic and Therapeutic Value of Liver Biopsies, New England J. Med. **237**:651-656 (Oct. 30) 1947.

MATERIAL AND METHODS

Sixty-three patients who entered the hospital because of acute alcoholism were observed. Two were studied on two separate occasions. With few exceptions these patients were also chronic alcoholics. In most patients there were no physical signs indicative of hepatic disease. Patients with ascites, hepatic coma or hematemesis were excluded.

The investigation included history, physical examination, a battery of liver function tests and liver biopsy. All biopsies were performed within 24 hours of admission, with three exceptions. Of these three biopsies, one was done within 48 hours and two within 72 hours. All patients were placed on a regular house diet which contained approximately 2,600 calories per day. They received no therapy other than paraldehyde sedation when necessary and penicillin when indicated. Biopsy was repeated in one patient after four days, in three patients after five days and in 46 patients after seven days. When 14 days had elapsed, a third biopsy was obtained in 14 patients.

The liver function tests were done in conjunction with the initial biopsy. They included determination of serum bilirubin,[3] thymol turbidity,[4] serum cholesterol and the cholesterol ester fraction,[5] serum alkaline phosphatase,[6] prothrombin time,[7] serum albumin and globulin partitions[8] and the 45 minute sulfobromophthalein (bromsulphalein®) sodium U. S. P. retention, with administration of 5 mg. of dye per kilogram of body weight. In addition, blood sugar and urea nitrogen levels, the blood Mazzini reaction, the hemogram and results of urinalysis were determined.

Prior to biopsy the patients received a sedative. Specimens were obtained with the Vim-Silverman needle, using the intercostal approach, and were fixed immediately in 4 per cent solution of formaldehyde. They were stained with hematoxylin and eosin. The initial site was used in subsequent biopsies. No complications were encountered in the 129 procedures performed. Examination of the tissue sections was done with no knowledge of the previous histologic observation or the clinical features.

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3. Malloy, H. T., and Evelyn, K. A.: Determination of Bilirubin with the Photoelectric Colorimeter, J. Biol. Chem. **119**:481-490 (July) 1937. Ducci, H., and Watson, C. J.: Quantitative Determination of the Serum Bilirubin with Special Reference to the Prompt Reacting and the Chloroform Soluble Types, J. Lab. & Clin. Med. **30**:293-300 (April) 1945.

4. Maclagen, N. F.: The Thymol Turbidity Test as an Indicator of Liver Dysfunction, Brit. J. Exper. Path. **25**:234-241 (Dec.) 1944.

5. Kingsley, G. R., and Schaffert, R. R.: Determination of Free and Total Cholesterol by Direct Chloroform Extraction, J. Biol. Chem. **180**:315-328 (Aug.) 1949.

6. Bodansky, A.: Phosphatase Studies: Determination of Serum Phosphatase Factors Influencing the Accuracy of the Determination, J. Biol. Chem. **101**:93-104 (June) 1933.

7. Aggeler, P. M.; Howard, J.; Lucia, S. P.; Clark, W., and Astaff, A.: Standardization of the Quick Prothrombin Time, Blood **1**:220-233 (May) 1946.

8. Gornall, A. G.; Bardawill, C. J., and David, M. M.: Determination of Serum Proteins by Means of the Biuret Reaction, J. Biol. Chem. **177**:751-766 (Feb.) 1949.

OBSERVATIONS

Fifty-five patients were men; eight were women. Fifty-seven were white; six were Negro. The ages ranged from 27 to 76 years.

The histories revealed that 61 patients were chronic alcoholics. The total period of alcoholism was given as under five years by nine patients, five to 10 years by eight, 10 to 20 years by 18 and over 20 years by 26. The length of the alcoholic bout which resulted in hospitalization was under one week in 23 cases, one to four weeks in 19 cases and over four weeks in 23 cases. When classified as to type of drinking, 31 patients were steady and 30 intermittent drinkers. The patient was considered a steady drinker if alcoholic consumption and dietary insufficiency were sustained over a long period. He was classified as an intermittent drinker if periods of alcoholic consumption and insufficient diet alternated with periods of normal diet and abstinence.

Two patients gave histories of acute alcoholism of one day's duration and gave no history of chronic alcoholism.

Nutrition was considered good in 24 cases, fair in 27 and poor in 14. Twenty-five patients showed specific evidence of avitaminosis. A palpable liver was recorded seven times. Spider angiomas were observed in four patients. Icterus was noted once. Physical examination did not reveal a palpable spleen, dilated collateral veins or liver palms in any case. Thirty-four patients had delirium tremens of mild to moderate degree during their hospital stay.

The results of liver function tests in 20 cases were within normal limits. In the remaining 45 the results of one or more tests were abnormal. These will be discussed more fully when they are compared with the histologic features.

In the group of 65 initial biopsies pathologic changes were present in 56 and absent in nine. The commonest abnormality was fatty metamorphosis, found 47 times. It was graded, according to severity, as slight in 10, moderate in seven, marked in 23 and extreme in seven. Fibrosis was present 13 times, was usually focal in distribution and was considered an early cirrhotic lesion. This lesion consisted of increased periportal fibrosis, usually associated with some inflammatory cell reaction and bile duct proliferation. The specimen obtained by needle biopsy was too small to enable one to see the nodular pattern of cirrhosis demonstrable in material obtained by other means, unless the cirrhosis was severe. Only once was it noted to be extensive. No attempt was made to grade this early cirrhosis as to severity. Other lesions observed were focal necroses in six, increased bile pigmentation in 14 and acute hepatitis and an unusual degree of hyperplasia in one each.

The second biopsy, obtained in 50 cases, revealed changes in 46 and no lesions in four. Fatty metamorphosis again was the commonest

lesion and was present in 28. It was slight in six instances, moderate in 11, marked in 10 and extreme in one. Cirrhotic changes were present in nine, focal necroses in four, increased bile pigmentation in seven and hepatitis and unusual hyperplasia in three each.

The third biopsy, obtained in 14 cases, revealed pathologic changes in all. Fatty metamorphosis was the most frequent. It was slight in four, moderate in two, marked in four and absent in four. None showed an extreme degree of fat. Cirrhosis was present in four, focal necroses in two and increased bile pigment and unusual hyperplasia in one each. Hepatitis was not present in any. The observations are summarized in table 1.

COMMENT

In following the histologic course of the liver in 50 alcoholic patients, we found the decrease in fat content the most prominent feature. Of the 42 patients whose biopsies revealed fatty metamorphosis initially, 32, or 76 per cent, showed some degree of fat reduction after one week. In six of these there was a three grade reduction, five changing from marked fatty metamorphosis to normal liver and one from extreme to slight fatty metamorphosis. Four patients showed a decrease of two grades, marked to slight in two and extreme to moderate and moderate to normal liver in one each. In 22 there was a one grade reduction, slight to normal in eight, moderate to slight in three, marked to moderate in seven and extreme to marked in four. Of the 10 remaining patients who showed no decrease in fat, eight displayed no change and two revealed a one degree increase.

Observations on the histologic course from the seventh to the fourteenth day in 14 patients failed to reveal as pronounced a fat decrease as observed after seven days. Four patients showed a one grade reduction and one a two grade reduction. Of the remaining nine, who showed no changes from the seventh to the fourteenth day, five failed to show fat reduction in the first week.

Disappearance of fat, with and without the administration of "lipotrophic" supplements, has been observed by others,[9] but the rapidity of fat reduction under controlled conditions has not been reported previ-

9. Volwiler, W.; Jones, C. M., and Mallory, T. B.: Criteria for the Measurement of Results of Treatment in Fatty Cirrhosis, Gastroenterology **11**:164-182 (Aug.) 1948. Beams, A. J., and Endicott, E. T.: Histologic Changes in the Livers of Patients with Cirrhosis Treated with Methionine, ibid. **9**:718-735 (Dec.) 1947. Franklin, M.; Salk, M.; Steigmann, F., and Popper, H.: Clinical, Functional, and Histological Responses of Fatty Metamorphosis of Human Liver to Lipotropic Therapy, Am. J. Clin. Path. **18**:273-282 (April) 1948. Klatskin, C., and Yesner, R.: Factors in the Treatment of Laennec's Cirrhosis of the Liver: I. Clinical and Histological Changes Observed During a Period of Bed Rest, Alcohol Withdrawal, and a Minimal Basic Diet, J. Clin. Investigation **28**:723-735 (July) 1949.

TABLE 1.—*Clinical, Functional and Histologic Observations on Alcoholic Patients**

Case No.	Age, Yr.	Sex	Race	Nutrition†	Avitaminosis	Palpable Liver	Spider "Nevi"	Jaundice	Liver Function Tests: Direct and Indirect Serum Bilirubin, Mg./100 Cc.	Thymol Turbidity, Units	Sulfobromophthalein Retention, %	Prothrombin Time, Seconds	Alkaline Phosphatase, Units	Serum Cholesterol and Cholesterol Esters, Mg./100 Cc.	Albumin and Globulin, Gm./100 Cc.	Initial Biopsy: Fat‡	Fibrosis	Necrosis	Hepatitis	Bile Pigment	Hyperplasia	Biopsy After 1 Wk.: Fat‡	Fibrosis	Necrosis	Hepatitis	Bile Pigment	Hyperplasia	Biopsy After 2 Wk.: Fat‡	Fibrosis	Necrosis	Hepatitis	Bile Pigment	Hyperplasia
1	50	F	W	G	0	0	0	0	0.1/1.0	5	2.5	13.8	1.8	240/184	4.2/2.5	1	0	0	0	X	0	0	0	0	0	X	0	..	..	..	..	..	..
2	35	F	W	G	X	0	0	0	0.2/0.8	4	4.0	12.2	2.8	230/208	3.5/2.8	2	0	0	0	0	0	0	0	0	0	0	X	..	..	..	..	..	..
3§	41	M	W	F	X	0	0	0	0.1/0.3	5	2.5	12.5	3.3	298/208	3.4/2.6	1	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..
4	46	M	W	F	0	0	0	0	0.1/0.6	3	4.0	13.0	2.4	180/128	3.0/2.6	2	X	0	0	0	0	1	X	0	0	0	0	..	..	..	..	..	..
5	45	M	W	G	0	0	0	0	0.3/0.6	3	2.8	12.4	4.0	208/141	4.1/2.6	1	0	0	0	0	X	0	0	0	0	0	X	..	..	..	..	..	..
6	65	M	W	F	0	0	0	0	0.3/0.6	3	2.0	14.0	2.4	190/136	3.2/2.4	1	0	0	0	X	0	0	0	0	0	0	0	..	..	..	..	..	..
7	41	M	W	F	X	0	0	0	0.1/0.3	3	4.0	13.2	4.0	216/152	3.9/2.2	0	0	0	0	X	0	0	0	0	0	0	0	..	..	..	..	..	..
8	50	M	W	G	0	0	0	0	0.3/1.2	2	3.2	12.4	2.4	320/192	4.0/2.0	3	0	0	0	0	0	2	0	0	0	X	0	..	..	..	..	..	..
9	58	M	W	G	0	0	0	0	0.4/0.6	1	3.2	11.0	2.8	216/161	3.5/1.7	3	0	X	0	0	0	0	0	0	0	X	0	..	..	..	..	..	..
10	65	M	W	P	0	0	0	0	0.4/1.6	2	4.8	11.4	4.2	326/230	3.6/2.5	3	0	X	0	0	0	2	0	0	0	0	0	..	..	..	..	..	..
11	56	M	W	P	0	0	0	0	0.1/0.4	4	0.0	13.2	3.9	280/202	3.4/2.9	3	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..
12	49	M	W	G	0	0	0	0	0.2/0.4	5	3.7	12.8	2.2	310/209	3.8/3.1	0	0	0	0	X	0	0	0	0	0	0	0	..	..	..	..	..	..
13‖	49	M	W	F	0	0	0	0	0.1/0.6	3	3.0	10.0	4.0	255/160	4.4/2.6	1	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..
14	60	M	W	G	X	0	0	0	0.4/1.4	4	6.0	12.0	1.8	300/193	5.0/3.7	3	0	0	0	0	0	2	0	0	0	0	0	..	..	..	..	..	..
15	37	M	W	F	X	0	0	0	0.5/1.2	4	3.2	14.0	3.6	237/192	5.4/3.1	4	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	0
16	60	M	W	P	0	0	0	0	0.2/1.0	5	3.2	12.4	3.2	158/107	4.1/3.1	1	X	0	0	0	0	0	0	0	0	0	0	0	X	X	0	0	0
17	42	M	W	F	X	0	0	0	0.1/0.2	4	6.0	12.5	3.0	165/120	4.5/3.0	3	0	0	0	0	0	2	0	0	0	0	0	1	X	0	0	0	0
18	34	M	W	P	X	0	0	0	0.3/1.0	5	2.0	12.6	4.7	163/128	4.7/2.7	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	63	M	W	F	X	0	0	0	0.2/0.5	2	14.0	10.0	4.8	258/210	3.9/3.5	4	0	0	0	0	0	3	0	0	0	0	0	3	X	X	0	0	0
20	64	M	W	F	X	0	X	0	0.1/0.4	2	8.0	12.0	3.2	266/168	4.7/4.1	4	0	0	0	0	0	3	0	0	0	0	0	2	X	0	0	0	0
21	39	M	N	F	0	0	0	0	0.4/1.4	3	12.0	13.2	5.0	200/121	3.3/2.4	3	0	0	0	0	0	2	0	0	0	0	0	..	..	..	..	..	..
22	41	M	W	F	0	0	0	0	0.1/0.3	2	5.0	11.6	1.9	200/146	2.3/1.2	3	X	X	0	X	0	0	0	0	0	X	0	..	..	..	..	..	..
23	64	M	W	F	0	X	0	0	0.2/0.7	4	11.8	12.2	4.0	226/152	3.6/2.6	4	X	0	0	0	0	2	0	0	0	0	0	..	..	..	..	..	..
24	72	M	W	F	X	X	X	0	0.2/0.7	4	26.0	12.0	4.6	220/140	3.4/3.0	1	0	X	0	0	0	0	X	X	0	X	0	..	..	..	..	..	..
25	35	M	N	F	0	X	0	0	0.1/0.6	9	7.6	13.0	3.3	242/174	2.4/2.9	2	0	0	X	0	0	1	X	0	0	0	0	..	..	..	..	..	..
26	40	M	W	F	0	0	0	0	0.3/0.6	2	8.0	13.4	5.4	190/132	4.5/2.0	4	X	0	0	0	0	3	X	0	0	0	0	..	..	..	..	..	..
27	76	M	W	F	X	0	0	0	3.1/7.0	9	42.0	14.0	5.8	204/142	2.9/2.8	3	0	0	0	0	0	2	X	0	0	0	0	..	..	..	..	..	..
28	60	F	W	P	X	0	0	0	0.3/0.8	3	16.8	12.2	2.9	182/62	4.0/4.3	4	0	0	0	0	0	3	0	0	0	0	X	..	..	..	..	..	..
29	67	F	W	P	X	0	0	0	0.5/1.2	6	4.0	15.0	6.5	280/166	4.0/2.3	0	0	0	0	X	0	0	0	0	0	0	0	..	..	..	..	..	..
30	49	M	W	P	X	0	0	0	0.2/0.7	3	2.6	13.6	5.2	168/116	3.4/4.4	2	0	0	0	X	0	1	0	X	0	0	0	..	..	..	..	..	..
31	48	M	W	P	X	0	0	0	0.5/1.0	2	6.0	11.0	5.6	280/210	4.9/2.4	1	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..

32	47	M	W	F	0	0	0	0	0.1/0.3	3	13.2	11.0	4.5	165/120	4.4/3.8	3	0	0	0	0	0	0	0	0	0	X	0	..	..	..	..	..	..
33	63	M	W	F	0	0	0	0	0.7/2.6	2	14.0	14.2	3.3	200/152	3.4/2.6	3	0	0	0	0	0	2	0	0	0	0	0	0	X	0	0	0	X
34	57	M	W	F	X	0	0	0	0.3/1.0	3	20.0	15.2	5.1	210/124	3.8/2.2	3	0	0	0	X	0	1	0	0	X	0	0	0	0	0	0	0	0
35	45	M	N	P	0	0	0	0	0.6/1.0	3	12.0	14.5	6.0	290/185	3.6/2.9	4	X	0	0	0	0	1	X	0	0	0	0	1	0	X	0	X	0
36§	41	M	W	G	X	0	0	0	0.1/0.4	2	0.0	12.2	5.7	200/77	4.3/3.4	3	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
37	75	M	W	G	0	0	0	0	0.5/1.2	2	3.2	14.0	2.8	245/200	4.8/3.7	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0
38	33	M	W	G	0	0	0	0	0.1/0.6	3	2.4	....	...	234/161	4.4/2.0	2	0	0	0	0	0	2	0	0	0	0	0#	..	..	..	..	..	..
39	33	F	W	F	0	0	0	0	0.2/0.7	7	15.0	11.8	7.0	252/168	4.6/4.0	3	X	0	0	0	0	3	X	0	X	0	0	..	..	..	..	..	..
40	32	M	N	G	0	0	0	0	0.3/0.6	3	7.0	....	3.7	322/200	4.6/3.0	3	0	0	0	0	0	3	0	0	0	0	0#	..	..	..	..	..	..
41	46	M	W	G	X	X	X	X	1.3/5.8	5	29.0	13.1	10.0	216/152	4.2/2.7	3	X	0	0	0	0	3	X	0	0	X	0	..	..	..	..	..	..
42	43	F	W	P	X	X	0	0	0.1/0.3	3	2.8	19.0	5.1	215/156	4.5/3.3	2	X	0	0	0	0	3	0	X	0	0	0	..	..	..	..	..	..
43	57	M	W	G	X	0	0	0	0.1/0.6	2	8.0	13.1	1.7	135/50	4.7/4.3	2	0	0	0	0	0	2	0	0	X	0	0	2	X	0	0	0	0
44	42	M	W	P	X	0	X	0	0.1/0.4	3	1.2	12.8	6.0	140/108	4.2/3.1	3	0	0	0	0	0	3	0	0	0	0	0	3	0	0	0	0	0
45	33	M	W	F	X	0	0	0	0.4/1.0	4	10.0	12.6	4.3	360/220	5.1/2.8	3	X	0	0	0	0	3	X	0	0	0	0	3	0	0	0	0	0
46	37	M	W	F	0	0	0	0	0.2/0.8	5	0.0	11.2	5.6	220/168	5.5/3.5	0	X	0	0	0	0	0	0	X	0	0	0	..	..	..	..	..	..
47	44	M	W	G	0	0	0	0	0.3/0.8	3	2.0	11.8	3.8	176/108	3.6/2.6	0	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..
48	55	M	W	G	0	0	0	0	0.2/0.8	2	6.0	12.0	4.2	246/176	3.9/2.3	0	0	0	0	0	0	0	0	0	0	0	0#	..	..	..	..	..	..
49	72	M	W	F	0	0	0	0	0.2/0.8	4	4.0	10.2	5.2	268/194	4.5/2.2	0	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..	..	..
50	50	M	N	G	0	0	0	0	0.3/0.7	1	3.2	11.0	3.8	224/172	5.0/3.3	0	0	0	0	0	0	0	0	0	0	0	0#	..	..	..	..	..	..
51	31	M	W	P	X	0	0	0	0.1/0.3	2	3.0	....	...	260/178	4.3/2.8	0	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
52	50	M	W	G	0	0	0	0	0.1/0.8	2	2.5	12.8	2.5	160/90	3.6/1.8	3	0	0	0	0	0	.	..	..	..	..	..	..	..	..	..	..	..
53	49	M	W	G	0	X	0	0	0.1/0.4	4	0.0	12.6	2.8	260/142	5.2/2.6	1	0	X	0	X	0	..	..	..	..	..	..	..	..	..	..	..	.
54	49	M	W	P	0	0	0	0	0.3/0.8	3	4.4	13.0	3.5	190/104	4.8/2.8	3	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
55	50	M	W	G	0	0	0	0	0.1/0.3	2	...	.....	...	200/144	4.9/2.6	0	0	0	0	X	0	..	..	..	..	..	..	..	..	..	..	..	..
56	51	M	W	G	0	X	0	0	.....	3	...	.....	...	.....	.....	3	X	0	0	X	0	..	..	..	..	..	..	..	..	..	..	..	..
57‖	49	M	W	G	0	0	0	0	0.1/0.2	3	13.0	.....	...	290/189	4.2/2.2	0	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
58	42	M	W	P	X	0	0	0	1.2/4.4	11	...	.....	...	130/84	4.6/3.8	0	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
59	38	M	W	F	X	0	0	0	0.1/0.4	6	2.6	12.2	3.2	270/165	5.0/3.2	0	0	0	0	0	0	..	.	..	..	..	..	..	..	..	..	..	..
60	[illegible]	F	N	F	0	0	0	0	0.2/0.6	6	4.0	12.0	3.9	160/113	3.7/3.6	0	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
61	48	M	W	G	0	0	0	0	0.3/0.7	8	0.0	12.0	3.7	210/144	3.7/2.9	0	0	0	0	X	0	..	..	..	..	..	..	..	..	..	..	..	..
62	..	M	W	F	0	0	0	0	0.2/0.8	2	14.0	11.2	4.0	160/104	3.3/2.3	0	0	X	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
63	31	M	W	G	0	0	0	0	0.1/0.6	..	0.0	11.6	6.0	210/175	4.2/2.0	0	X	0	0	X	0	..	..	..	..	..	..	..	..	..	..	..	..
64	37	M	W	G	0	0	0	0	0.9/2.6	7	...	12.8	2.0	282/196	4.3/2.5	0	0	0	0	X	0	..	..	..	..	..	..	..	..	..	..	..	..
65	49	F	W	F	0	0	0	0	0.5/2.6	5	2.0	13.6	3.2	170/70	4.1/3.4	3	0	0	0	0	0	..	..	..	.	.	..	..	.	..	..	..	..

* X indicates presence and 0 absence of findings.

† Nutrition is graded G, good; F, fair, and P, poor.

‡ Fatty metamorphosis is graded as follows: 1, slight; 2, moderate; 3, marked, and 4, extreme.

§ Cases 3 and 36 are the same patient, observed on two separate occasions.

‖ Cases 13 and 57 are the same patient, observed on two separate occasions.

¶ Biopsy repeated after four days.

Biopsy repeated after five days.

ously. No common denominator was found which could be applied to those patients showing fat disappearance or those whose condition failed to improve. It is obvious that the existing degree of fatty change in alcoholic persons during periods of alcoholic intake and dietary insufficiency can be properly estimated only if the biopsy material is obtained before normal diet is resumed. Any estimate of the degree of fatty metamorphosis in these patients from biopsy specimens obtained as little as one week after resumption of normal diet may be entirely misleading. Further, no evaluation of the efficacy of various substances reputed to have a lipotropic effect can be valid unless the effect of a normal diet is first observed.

The second feature of interest was the cirrhosis which was seen in 21 patients. With one exception it was a focal lesion and not a diffuse disease. The focal nature of this lesion, as well as the small amount of tissue provided by needle biopsy, probably explains the fact that cirrhosis was not observed consistently in biopsies of individual cases. It

TABLE 2.—*Comparison of Serial Biopsies in Patients with Cirrhosis**

No. of Patients	Initial Biopsy	Biopsy After 1 Wk.	Biopsy After 2 Wk.
4	X	X	..
3	X	0	..
4	0	X	..
5	0	0	X
2	X	X	0
1	X	0	X

* X indicates the presence of cirrhosis and 0 its absence.

is true that a marked degree of fatty change may obscure increased fibrosis and that, following reduction in the amount of fat, cirrhosis may become evident. It was noted, however, that early cirrhotic changes may be absent in an individual biopsy specimen completely devoid of fat. We have seen in several patients with five biopsies taken at weekly intervals that cirrhosis was not evident in the first four biopsies and was discovered in the fifth.[10] Other investigators have distinguished between diffuse and focal pathologic involvement of the liver with regard to interpreting needle biopsy material.[1b] The results summarized in table 2 clearly illustrate that the danger of missing existing pathologic alterations in one or more needle biopsy specimens applies as well to early fibrotic changes as to other focal lesions.

The physical examination was of little aid in detecting the presence of hepatic disease. This is understandable when the paucity of advanced hepatic lesions is considered. Patients with histologic evidence of cirrhosis included six of the seven with palpable livers, three of the four with spider angiomas and the one patient with clinical icterus. Among

10. Seife, M.; Kessler, B. J., and Lisa, J. R.: Unpublished data.

the patients with cirrhosis 17 were steady and four were intermittent drinkers. This preponderance of steady drinkers in cirrhosis has been reported elsewhere.[2a]

Focal necroses were observed 12 times and in individual cases with serial biopsies showed the same lack of consistency as did the cirrhosis. Necrotic foci were not consistently associated with any other histologic abnormality, and there were no clinical features to suggest their presence.

The results of other workers who have correlated laboratory and histologic findings are not in agreement.[11] We made no attempt to draw any conclusions with regard to a correlation of all the laboratory and biopsy data, nor did we attempt to compare the severity of histologic and functional changes. It is noteworthy, however, that 16 of the 21 patients with cirrhosis showed definitely abnormal results in one or more liver function tests, despite the early nature of the cirrhosis found. Increased sulfobromophthalein retention and derangement of the albumin and globulin partitions were the commonest abnormalities associated with cirrhosis. In 36 per cent of the cirrhotic patients the lesion was not observed in the initial biopsy but was discovered in subsequent specimens. The results of the laboratory tests in these cases, done in conjunction with the initial biopsy, were considered definitely abnormal. In three patients with cirrhosis the functional results were equivocal, and in two they were normal.

When the initial histologic observations on those patients who never showed cirrhosis were compared with the laboratory results, no degree of correlation was found. Of 21 cases in which the results on laboratory tests were considered normal, biopsy revealed normal liver in five, fatty metamorphosis alone or in combination with lesions other than cirrhosis in 14 and increased bile pigmentation in two. In 23 cases the laboratory results were considered abnormal, and of these a normal liver was found in four, fatty metamorphosis alone or in combination with a lesion other than cirrhosis in 14, focal necrosis in one and increased bile pigmentation in four.

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11. Popper, A.; Steigmann, F.; Meyer, K. A.; Kozoll, D., and Franklin, M.: Correlation of Liver Function and Liver Structure, Am. J. Med. **6**:278-291 (March) 1949. Kinsell, L. N.; Weiss, H. A.; Michaels, G. D.; Shaver, J. S., and Barton, H. C.: The Correlation of Hepatic Structure and Function, ibid. **6**:292-308 (March) 1949. Popper, H.; Franklin, M.; Steigmann, F., and Kozoll, D.: Relationship Between Structural and Functional Alterations of the Liver, J. Lab. & Clin. Med. **32**:318-319 (March) 1947. Sherlock, S. P. V.: Biological Investigations in Liver Disease: Some Correlation with Hepatic Histology, J. Path. & Bact. **58**:523-544 (July) 1946. Post, J., and Rose, J. V.: Clinical, Functional, and Histologic Studies in Laennec's Cirrhosis of the Liver, Am. J. Med. **8**:300-313 (March) 1950. Mateer, J. G.; Hartman, F. W.; Baltz, J. L.; Fallis, L. D.; McGraw, A. B., and Steele, H. H.: Combined Liver Biopsy and Liver Function Study in 132 Cases of Cholelithiasis and 31 Cases of Peptic Ulcer, Gastroenterology **11**:284-300 (Sept.) 1948.

REPORT OF CASES

Four cases are described because of their illustrative value or because of special interest.

CASE 34.—A 57 year old white male park worker was admitted to the hospital because of acute alcoholism (table 1). For nine months prior to his admission he had been consuming large quantities of wine daily, and his diet had been deficient. Before this, he was a moderate beer drinker and his diet was normal. Examination revealed a poorly nourished man with scabies and smooth hyperemic tongue edges. The abnormal results of laboratory tests performed on admission were a 20 per cent cent retention of sulfobromophthalein and a prothrombin time of 15 seconds (normal, 10 to 14 seconds). Liver biopsy on admission revealed extreme fatty meta-

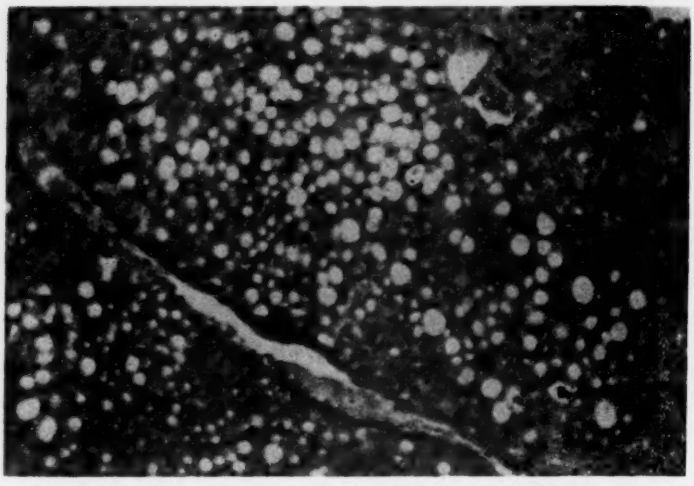

Fig. 1 (case 34).—Marked fatty metamorphosis of liver present on initial biopsy; × 100.

morphosis and increased bile pigmentation. Shortly after admission he had mild delirium tremens, but his condition improved rapidly, and after two days he was able to take the house diet. The only medicaments received by the patient during 17 days of hospitalization were 10 cc. of paraldehyde U. S. P. and a sedative prior to biopsy. A liver biopsy obtained after one week showed mild fatty metamorphosis and slight round cell infiltration. The sulfobromophthalein retention at this time was 15.6 per cent. At the end of two weeks, a third liver biopsy showed only slight fatty metamorphosis and the sulfobromophthalein retention was 6.0 per cent.

Comment.—This case is representative of those showing histologic improvement. At the same time, in this case, there was corresponding functional improvement.

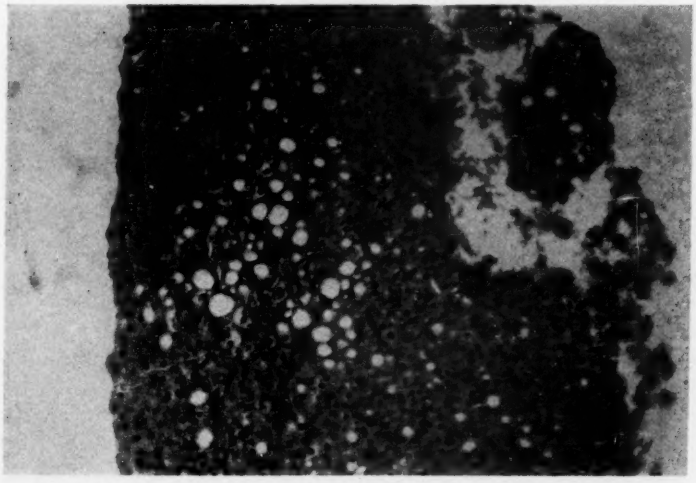

Fig. 2 (case 34).—Moderate amount of fat on second biopsy taken one week later; × 100.

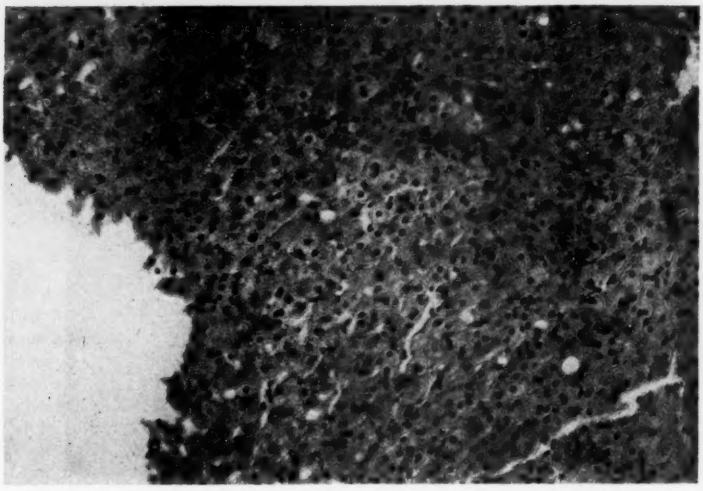

Fig. 3 (case 34).—Normal appearance of liver on biopsy taken at end of 14 days; × 100.

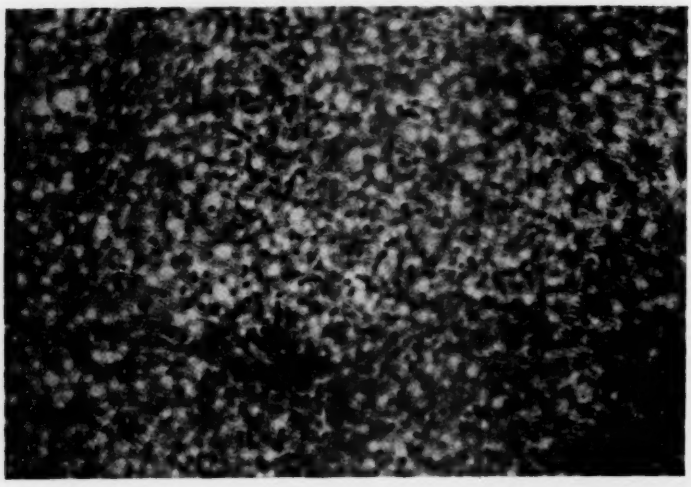

Fig. 4 (case 35).—Marked fatty metamorphosis of liver on initial biopsy; × 100.

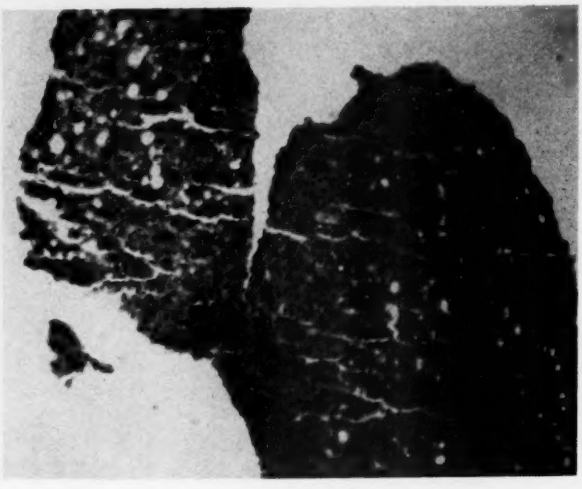

Fig. 5 (case 35).—Great reduction of fat and evidence of cirrhosis found in liver at end of one week; × 100.

CASE 44.—A 42 year old white man, employed as a ship's radio operator, was admitted to the hospital because of acute alcoholism (table 1). He stated that for one week before admission he had been drinking large amounts of whisky and small amounts of beer each day. During this time his dietary intake had been almost nothing. He had been an intermittent drinker since the age of 15. Examination revealed a beefy red tongue and skin lesions suggestive of vascular spiders. The only abnormal laboratory finding was an alkaline phosphatase value of 6.0 Bodansky units. Liver biopsy on admission revealed marked fatty metamorphosis. The patient ate well during his hospital stay. The only medicament he received was a sedative prior to biopsy. Repeated biopsy after seven and 14 days disclosed marked fatty metamorphosis on both occasions.

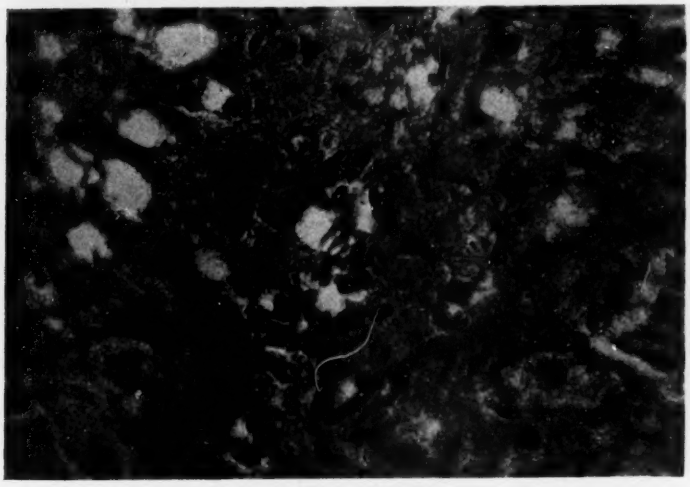

Fig. 6 (case 35).—Focal necroses; × 450.

Comment.—This case is representative of the group which showed no change in the degree of fatty metamorphosis.

CASE 36.—This patient was studied on two occasions (table 1). When first seen, this 41 year old white man, a laborer, was intoxicated. He had been drinking 3 quarts (2.8 liters) of fortified wine daily for six days and stated that he had eaten nothing during this time. The past history was one of intermittent drinking for 22 years. Examination revealed a beefy red tongue. The results of the battery of liver function tests were considered normal. Liver biopsy on admission disclosed slight fatty metamorphosis. During the first hospital day the patient required 6 cc. of paraldehyde. This and the sedative administered before biopsy were the only medicaments he received. Biopsy after one week revealed normal liver, and the patient was discharged. He was readmitted two months later because of acute alcoholism and pediculosis. He had been consuming large amounts of fortified wine daily for four weeks and had eaten nothing during this time. Physical examination revealed a beefy red tongue, scabies, skin pustules and pediculi.

Results of liver function tests were again normal. Liver biopsy revealed marked fatty metamorphosis. During the first two hospital days the patient required paraldehyde sedation and was unable to eat. His appetite then improved, and he ate a house diet. He received no specific therapy. Biopsies after seven and 14 days showed slight fatty metamorphosis.

CASE 13.—A 49 year old white man, a salesman, was admitted to the hospital because of acute alcoholism (table 1). He stated that every day for one month he had been drinking enough whisky and ale to maintain a constant state of euphoria. His diet during this time had been poor. Results of physical examination, laboratory tests and liver biopsy on admission were normal. He had moderately severe delirium tremens; his condition improved in three days, and he left the hospital. He was readmitted in one month, again acutely alcoholic. He stated that he had been constantly drunk on whisky and ale since leaving the hospital. He had eaten nothing during this time. Results of physical examination and liver function tests were normal. Liver biopsy on admission revealed slight fatty metamorphosis. After the patient received the house diet for one week, the biopsy was repeated and disclosed normal liver.

Comment.—These two cases illustrate changes in the degree of fatty metamorphosis which took place while the patients were observed on two separate occasions.

SUMMARY AND CONCLUSIONS

Sixty-three alcoholic patients were observed clinically, functionally and histologically at the time of alcoholic withdrawal and resumption of normal diet. Serial biopsies were done on a number of patients after one and two weeks. A rapid disappearance of fat was noted in most cases of fatty metamorphosis despite the absence of specific therapy.

Early in its progress, cirrhosis was found to have a focal distribution and because of this a high degree of inconsistency was observed in the serial biopsies in cirrhotic patients. Most patients with cirrhosis fell into the classification of steady drinkers. In patients showing early cirrhosis, the sulfobromophthalein sodium retention and the albumin to globulin partitions were frequently noted to be abnormal. In patients showing lesions other than cirrhosis, no correlation between morphologic changes and liver function was found.

Four cases of illustrative value or special interest are described in detail

USE OF CHOLINE SUPPLEMENTS IN FATTY METAMORPHOSIS OF THE LIVER

A Needle Biopsy Investigation in Human Beings

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NEW YORK

ALLAN and his co-workers [1] observed that depancreatized dogs kept alive with insulin acquired fatty livers and that the change was preventable by the oral administration of raw beef pancreas. Hershey [2] and Hershey and Soskin [3] later reported that lecithin could be substituted for raw beef pancreas. In 1932, Best and his associates [4] identified choline as the active principle in lecithin. Choline is effective in preventing experimentally produced cirrhosis in laboratory animals, as has been shown by György and Goldblatt [5] and others.[6] The successful thera-

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1. Allan, F. N.; Bowie, D. J.; MacLeod, J. J. R., and Robinson, W. L.: Behavior of Depancreatized Dogs Kept Alive with Insulin, Brit. J. Exper. Path. **5**:75-83 (April) 1924.

2. Hershey, J. M.: Substitution of Lecithin for Raw Pancreas in the Diet of the Depancreatized Dog, Am. J. Physiol. **93**:657-658 (June) 1930.

3. Hershey, J. M., and Soskin, S.: Substitution of "Lecithin" for Raw Pancreas in the Diet of the Depancreatized Dog, Am. J. Physiol. **98**:74-85 (Aug.) 1931.

4. Best, C. H.; Hershey, J. M., and Huntsman, M. E.: The Effect of Lecithine on Fat Deposition of the Liver of the Normal Rat, J. Physiol. **75**:56-66 (May) 1932. Best, C. H., and Huntsman, M. E.: The Effects of the Components of Lecithine upon Deposition of Fat in the Liver, ibid. **75**:405-412 (Aug.) 1932.

5. György, P., and Goldblatt, H.: Observations on the Conditions of Dietary Hepatic Injury (Nephrosis, Cirrhosis) in Rats, J. Exper. Med. **75**:355-367 (April) 1942.

6. Daft, F. S.; Sebrell, W. H., and Lillie, R. D.: Production and Apparent Prevention of a Dietary Liver Cirrhosis in Rats, Proc. Soc. Exper. Biol. & Med. **48**: 228-229 (Oct.) 1941; Prevention by Cystine or Methionine of Hemorrhage and Necrosis of the Liver in Rats, ibid. **50**:1-5 (May) 1942. Blumberg, H., and McCollum, E. V.: The Prevention by Choline of Liver Cirrhosis in Rats on High Fat, Low Protein Diets, Science **93**:598-599 (June) 1941. Handler, P., and Dubin, I. N.: The Significance of Fatty Infiltration in the Development of Hepatic Cirrhosis Due to Choline Deficiency, J. Nutrition **31**:141-157 (Feb.) 1946.

peutic use of choline in human cirrhosis has been reported in several communications.[7] Patek [8] and others,[9] however, pointed out factors in these investigations, such as the absence of control patients, insufficient control periods in the patients treated and the small number of patients observed, which make their evaluation hazardous. Recently, the use of the needle biopsy of the liver has made possible histological, as well as clinical, evaluation of various agents in the treatment of hepatic disease. The histological studies of the effectiveness of lipotropic supplements have not been in agreement. Beams and Endicott [10] and Franklin and his co-workers [11] concluded from their observations that lipotropic supplements, including choline, choline and cystine and methionine, were responsible for the improvement noted. Other workers [12] reported similar improvement without the use of lipotropic supplements.

During the investigations on 149 alcoholic and tuberculous patients, it was observed that in a certain number diet or diet with multivitamin supplements failed to produce any appreciable change in fatty metamorphosis of the liver. Among this group eight patients remained under

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7. Broun, G. O., and Muether, R. O.: Treatment of Hepatic Cirrhosis with Choline Chloride and Diet Low in Fat and Cholesterol, J. A. M. A. **118**:1403 (April 18) 1942. Russakoff, A. H., and Blumberg, H.: Choline as an Adjuvant to the Dietary Therapy of Cirrhosis of the Liver, Ann. Int. Med. **21**:848-862 (Nov.) 1944. Beams, A. J.: The Treatment of Cirrhosis of the Liver with Choline and Cystine, J. A. M. A. **130**:190-194 (Jan. 26) 1946. Morrison, L. M.: The Response of Cirrhosis of the Liver to an Intensive Combined Therapy, Ann. Int. Med. **24**: 465-478 (March) 1946. Steigmann, F.: Efficacy of Lipotropic Substances in Treatment of Cirrhosis of Liver, J. A. M. A. **137**:239-242 (May 15) 1948.

8. Patek, A. J., Jr.: An Evaluation of Dietary Factors in the Treatment of Laennec's Cirrhosis of the Liver, J. Mt. Sinai Hosp. **14**:1-7 (May-June) 1947.

9. Chalmers, T. C., and Davidson, C. S.: A Survey of Recent Therapeutic Measures in Cirrhosis of the Liver, New England J. Med. **240**:449-455 (March 24) 1949.

10. Beams, A. J., and Endicott, E. T.: Histologic Changes in the Livers of Patients with Cirrhosis Treated with Methionine, Gastroenterology **9**:718-735, 1947.

11. Franklin, M.; Salk, M.; Steigmann, F., and Popper, H.: Clinical, Functional and Histologic Responses of Fatty Metamorphosis of Human Liver to Lipotropic Therapy, Am. J. Clin. Path. **18**:273-282 (April) 1948.

12. Volwiler, W.; Jones, C. M., and Mallory, T. B.: Criteria for the Measurement of Results of Treatment in Fatty Cirrhosis, Gastroenterology **11**:164-182 (Aug.) 1948. Buck, R. E.: Observations on Fatty Alcoholic Liver: The Use of Interval Needle Biopsy and Liver Function Tests, J. Lab. & Clin. Med. **33**:555-564 (May) 1948. Chalmers, T. C.; Murphy, T. L., and Taft, E. B.: Incidence, Character and Course of Liver Disease in Chronic Alcoholics as Determined by Needle Biopsy, J. Clin. Investigation **27**:528-529 (July) 1948. Klatskin, C., and Yesner, R.: Factors in the Treatment of Laennec's Cirrhosis of the Liver: Clinical and Histological Changes Observed During a Period of Bed Rest, Alcohol Withdrawal, and a Minimal Basic Diet, ibid. **28**:723-735 (July) 1949.

observation a sufficient length of time to enable a study to be carried out on the effect of choline supplements as a lipotropic agent. These cases form the basis of this communication. The methods of study have been previously reported.[13] Five patients were placed on a 2,800 calory diet alone, and three were on a 3,400 calory diet with multivitamin supplements. After an initial control period, choline chloride in daily doses of 3 to 9 Gm. was administered orally. The histological course was then observed at weekly intervals for two to four weeks. The degree of fatty metamorphosis was graded histologically according to severity into four categories, mild, moderate, marked and extreme.

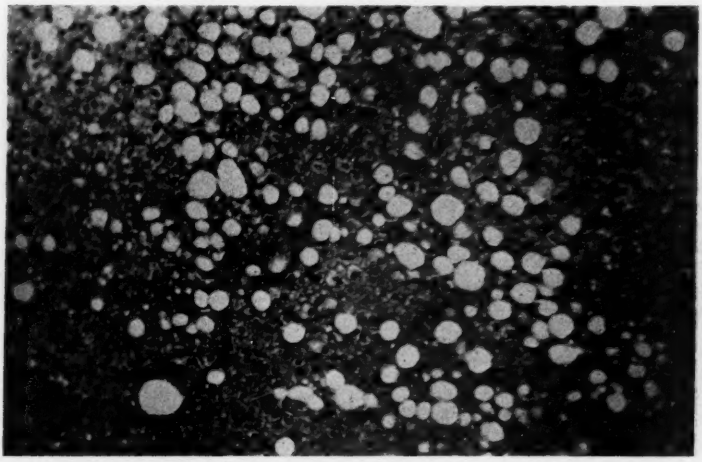

Fig. 1 (case 1).—Biopsy 1, taken the day of admission, showing marked fatty metamorphosis.

REPORT OF CASES

CASE 1.—A 33 year old white laborer was admitted to the hospital after a particularly heavy two week alcoholic bout. He had eaten no food during this period. The history revealed that he was a chronic, steady drinker. Physical examination on his admission disclosed early delirium tremens and signs of avitaminosis; the other findings were normal. Biopsy revealed marked fatty metamorphosis and several foci of necrosis. It was necessary to give this patient several doses of paraldehyde. He was placed on the house diet and received no other medication.

13. Seife, M.; Kessler, B. J., and Lisa, J. R.: Clinical, Functional and Needle Biopsy Study of the Liver in Alcoholism, Arch. Int. Med., this issue, p. 658. A Clinical, Functional and Needle Biopsy Study of the Liver in Pulmonary Tuberculosis, Am. Rev. Tuberc., to be published.

Tremors subsided; appetite improved, and within three to four days he was eating all the food placed before him. After one week, biopsy revealed marked fatty metamorphosis and cirrhosis. On the fifteenth hospital day the biopsy showed no change. On this day administration of choline chloride, 3 Gm. daily, was begun. Biopsies obtained on the twenty-second and twenty-ninth days showed marked fatty metamorphosis.

CASE 2.—A 63 year old butcher was admitted to the hospital after eight months of steady alcoholism and dietary insufficiency. Examination revealed alcoholic tremors, absence of chest and axillary hair and signs of avitaminosis. Biopsy revealed extreme fatty metamorphosis. The tremors subsided; appetite improved, and within two to three days he was eating all the food placed before him. Biopsies on the ninth and sixteenth hospital days showed marked fatty metamorphosis.

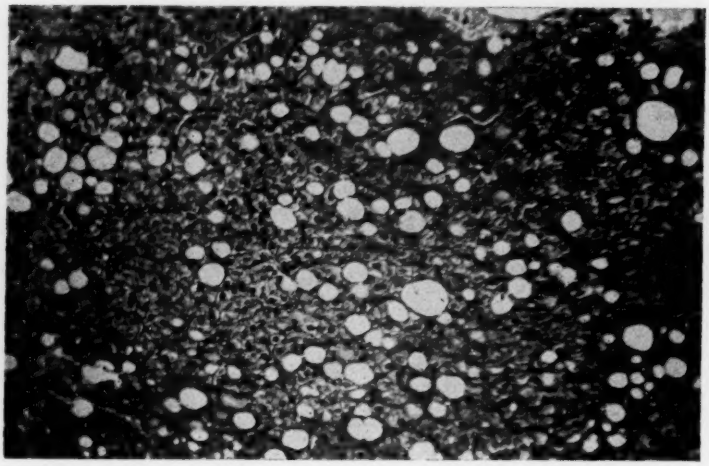

Fig. 2 (case 1).—Biopsy 3, taken on fifteenth day after two weeks of house diet, showing no change in fat content.

Administration of choline chloride, 3 Gm. daily, was started on the sixteenth day. Moderate fatty metamorphosis was noted after one week of choline therapy. Two weeks after the institution of choline treatment, biopsy showed marked fatty metamorphosis. Cirrhosis was observed in the fifth biopsy.

CASE 3.—A 64 year old steady wine drinker was admitted to the hospital because of skin lesions on upper and lower extremities suggestive of pellagra. His diet had been extremely poor for one year. In addition to skin lesions, there were changes of avitaminosis in the tongue, several spider angiomas on the chest and sparsity of body hair. The patient was placed on the routine house diet, which he ate well during his entire hospital stay. He received no specific therapy during the first two weeks. Biopsies done on the second, ninth and sixteenth hospital days revealed extreme, marked and moderate fatty metamorphosis, respectively. Treatment with choline chloride, 3 Gm. daily, was started on the sixteenth day.

Biopsies on the twenty-third and thirtieth days revealed moderate fatty metamorphosis. Cirrhosis was present in the fourth and fifth biopsies only. The pellagrous lesions cleared completely, despite the absence of specific vitamin therapy.

CASE 4.—A 53 year old female cook entered the hospital because of anorexia. The history revealed chronic, steady alcoholism for 5½ years and a diet of one meal and 1 quart (0.9 liter) of milk daily. The results of physical examination were completely normal. The patient was placed on the routine house diet and during the entire hospital stay ate all the food placed on her tray. A biopsy obtained on the second hospital day revealed extreme fatty metamorphosis, cirrhosis and foci of necrosis. Biopsies obtained on the ninth and sixteenth days showed marked fatty metamorphosis and cirrhosis. Administration of choline chloride, 6 Gm. daily, was instituted on the sixteenth hospital day. On the thirtieth day, after two weeks

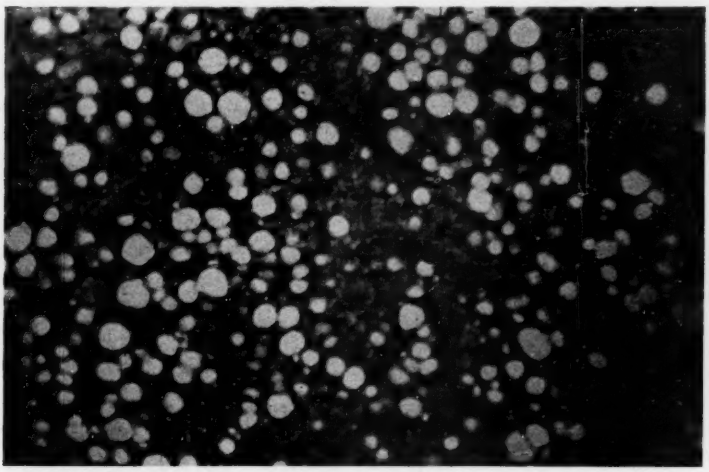

Fig. 3 (case 1).—Biopsy 5, taken on twenty-ninth day after administration of 42 Gm. of choline in 14 days, showing practically unchanged fat content.

of choline therapy, biopsy revealed marked fatty metamorphosis, cirrhosis and foci of necrosis.

Comment.—These four cases are similar in the following respects: 1. The patients were chronic, steady drinkers and gave histories of sustained dietary insufficiency. 2. They presented no physical signs pointing specifically to hepatic disease. 3. Definite, although early, cirrhosis was present in one or more biopsies in each patient. 4. The clinical courses were much the same in that there was rapid recovery from postalcoholic tremors and gastrointestinal symptoms. Within a few days of their admission, the patients were eating all the food placed on their trays. In all the cases there was an over-all reduction in fat, varying in degree, which could be seen by comparing the first and last biopsies. In none of the cases, however, was there a significant acceleration in the clearance of fat after the institution of choline therapy.

CASE 5.—A 37 year old doorman was admitted to the hospital because of early delirium tremens, following a five day bout of alcoholism. The history revealed intermittent drinking over an eight year period. Physical examination disclosed the lingual signs of avitaminosis. The patient required large amounts of sedatives during the first week in the hospital. His appetite improved slowly, and after seven to eight days he was able to eat all the food placed on his tray. On the second hospital day biopsy revealed extreme fatty metamorphosis. On the ninth day biopsy showed the same degree of fatty change. On the fifteenth day, biopsy revealed some diminution in fat and the degree of change was graded as marked fatty metamorphosis. The patient was discharged as improved.

He was readmitted 11 weeks later, after a six day bout of alcoholism and dietary insufficiency. Examination again revealed pronounced tremors and signs of avitaminosis. During the first two weeks in the hospital the patient required large amounts

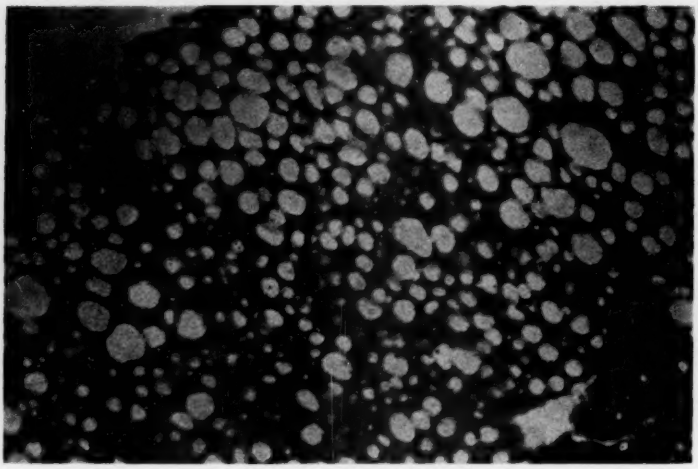

Fig. 4 (case 4).—Biopsy 1, taken on second hospital day, showing extreme fatty metamorphosis.

of sedatives. His appetite improved slowly, and after eight to nine days he was eating all the food placed before him. Biopsies obtained on the third and tenth hospital days revealed extreme fatty change. On the seventeenth day the fatty change was graded as marked. On this day administration of choline chloride, 9 Gm. daily by mouth, was started. Biopsies on the twenty-fourth and thirtieth days showed moderate and slight fatty change, respectively. Cirrhosis was evident on the sixth and eighth biopsies only.

Comment.—This case is similar to the first four in most respects. The patient, however, on both hospital admissions made a slow clinical recovery from his post-alcoholic state. On both occasions it was necessary to use heavy sedation, and he was not eating properly until after a week in the hospital. In this case a significant acceleration in the clearing of fat occurred after the institution of choline therapy. It is noteworthy that in his two hospital admissions a total of eight needle biopsies was obtained from this patient without any untoward effects.

CASE 6.—A 38 year old Puerto Rican woman was admitted to the hospital because of cough, expectoration, loss of weight and pain in the chest. History revealed that for many years she ate only one good meal and drank large quantities of beer each day. Examination showed bilateral signs of tuberculosis, confirmed by roentgenography and sputum examination. The liver edge was palpable 6 cm. below the right costal margin and was smooth and nontender. The patient was placed on strict rest in bed and a 3,400 calory diet, with multivitamin supplements. Her appetite was excellent, and she ate all the food placed on her tray. After one week she was given dihydrostreptomycin, 1 Gm., and paraaminosalicylic acid, 10 Gm. daily. Biopsies obtained on the second, ninth and sixteenth hospital days showed cirrhosis, foci of necrosis and fatty metamorphosis, in each instance graded as extreme. Treatment with choline chloride, 9 Gm. daily, was started on

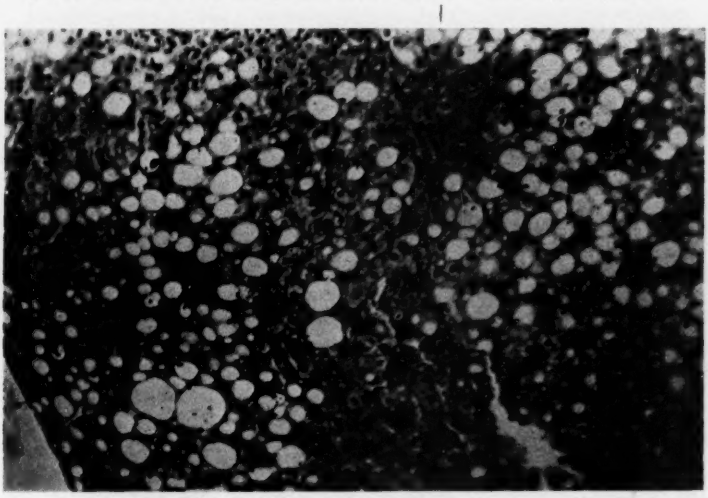

Fig. 5 (case 4).—Biopsy 3, taken on sixteenth day after two weeks of house diet, showing marked fatty metamorphosis.

the sixteenth day. On the twenty-third and thirtieth days biopsies showed cirrhosis and fatty change, both graded as marked.

Comment.—This patient showed a slight reduction in fat over a period of four weeks. Although the degree of fatty metamorphosis changed from the objective category of extreme to marked after one week of choline administration, a comparison of biopsies in this case did not show a significant increase of fat clearance after the institution of choline therapy.

CASE 7.—A 56 year old white man entered the hospital because of night sweats, hemoptysis and a 20 pound (9.1 Kg.) weight loss in eight months. The chest roentgenogram revealed bilateral, far advanced tuberculosis, and the sputum contained tubercle bacilli. The patient also complained of anorexia with resultant poor food intake. He was placed on strict rest in bed and a 3,400 calory diet, with multivitamin supplements. Shortly after his admission he was given dihydrostreptomycin, 1 Gm., and paraaminosalicylic acid, 10 Gm. daily. His condition improved

slowly on this regimen, and he was able to eat most of the food placed on his tray. Biopsy obtained on the thirty-first hospital day revealed marked fatty metamorphosis, several foci of necrosis and prominent bile pigmentation. Administration of choline chloride, 9 Gm. daily, was started on the thirty-fourth hospital day. Biopsy on the forty-first day showed marked fatty change and on the forty-eighth day disclosed moderate fatty change, foci of necrosis, periportal inflammation and conspicuous bile pigmentation. On the sixty-second day, one month after the institution of choline therapy, biopsy revealed moderate fatty change, a periportal tubercle, foci of necrosis and periportal inflammation.

CASE 8.—A 51 year old Puerto Rican woman entered the hospital because of chills, fever, cough and pain in the chest of two weeks' duration. In the six months before her entry she had lost 15 pounds (6.8 Kg.). The chest roentgenogram revealed bilateral, moderately advanced tuberculosis, and the sputum contained

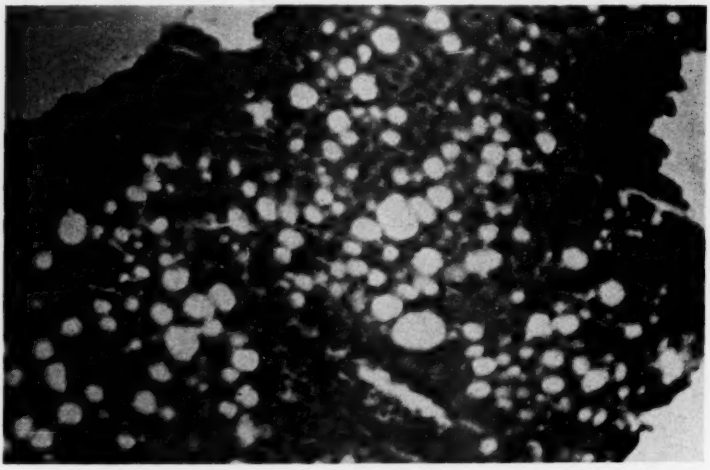

Fig. 6 (case 4).—Biopsy 5, taken on thirtieth day after administration of 84 Gm. of choline in 14 days, showing unchanged fat content.

tubercle bacilli. She was placed on strict rest in bed and given a 3,400 calory diet, multivitamin supplements and dihydrostreptomycin, 1 Gm., and paraaminosalicylic acid, 10 Gm. daily. On this regimen she showed steady improvement. On the forty-seventh hospital day a liver biopsy revealed moderate fatty metamorphosis and several foci of necrosis. Treatment with choline chloride, 9 Gm. daily, was started on the fifty-third hospital day. On the sixtieth day, biopsy showed moderate fatty metamorphosis and several foci of necrosis, and, on the sixty-seventh day, slight fatty metamorphosis, cirrhosis, foci of necrosis and periportal inflammation. On the eighty-first day, one month after the addition of choline, biopsy still showed slight fatty metamorphosis and several foci of necrosis.

Comment.—A reduction from marked to moderate and moderate to slight fatty change, respectively, was noted in the two patients described above. Both were observed for one month, during which choline chloride, 9 Gm. daily, was administered.

COMMENT

The results presented indicate that, in persons with fatty change of the liver due to the malnutrition associated with alcoholism or tuberculosis, choline supplements offer no particular value over proper diet alone in clearing fat from the liver. It was felt that the early nature of the cirrhosis in the patients treated with choline offered an opportunity to study the effect of this substance more critically than has been done in other investigations. The diagnosis of cirrhosis was made histologically, while there were no clinical indications of the disease. It was not necessary to treat these patients with an intensive combined regimen [14]; instead they were placed on the routine house diet. Thus, the evaluation is not complicated by a multiplicity of clinical abnormalities or therapeutic measures and concerns the use of a routine house diet with and without the addition of choline. The results have been measured histologically with serial liver biopsies.

Clearance of fat from the liver of 66 alcoholic patients on the house diet alone varied in degree. The majority, however, showed an appreciable reduction in fat, and in many the rate of reduction was exceedingly rapid. These changes were noted in two weeks after the withdrawal of alcohol and the resumption of normal diet. Among these patients were 25 whose biopsies showed early cirrhosis. In 20 of them a significant reduction in fat was seen after one or two weeks. In the remaining 5 the fat content was unchanged. Patients 1, 2, 4 and 5 (table 1) were among those in whom little, if any, reduction in fat content occurred during the two weeks of observation on a 2,800 calory diet. Patients 1, 2 and 4 had no acceleration in the fat clearance after the addition of daily oral doses of choline chloride. Patient 5 showed acceleration in fat clearance after choline was added. In patient 3 (table 1) a considerable reduction in fat content took place during the two week observation period on diet alone. The degree of clearance in this patient during the two weeks of choline administration was actually less than during the control period. It is realized that the control periods were short. This fact makes the response in patient 5 difficult to evaluate but is of no consequence with regard to the lack of response seen in the first four cases.

Patient 6 is another with a lack of significant response after the addition of choline and is considered separately because of the presence of pulmonary tuberculosis, as well as alcoholism. Among the 149 patients studied there were two other instances of fatty metamorphosis occurring in alcoholic patients with pulmonary tuberculosis. In both these patients

14. Patek, A. J., Jr., and Post, J.: Treatment of Cirrhosis of Liver by Nutritious Diet and Supplements Rich in Vitamin B Complex, J. Clin. Investigation **20**:481-505 (Sept.) 1941.

TABLE 1.—*Summary of Observations in Six Patients Treated with Choline*

Patient No.	1	2	3	4	5	6
Age, yr.	33	63	64	53	38	38
Sex	M	M	M	F	M	F
Race	W	W	W	W	W	W
Pertinent results of functional tests	Sulfobromophthalein retention, 10%	Sulfobromophthalein retention, 14.2%	Sulfobromophthalein retention, 8.0%; albumin, 4.7 Gm.; globulin, 4.1 Gm.	Sulfobromophthalein retention, 8.0%	Bilirubin, 0.4 mg. (direct); 1.6 mg. (indirect)	Bilirubin, 0.9 mg. (direct) and 1.8 mg. (indirect); albumin, 3.3 Gm.; globulin, 4.7 Gm.; thymol turbidity, 6 units; alkaline phosphatase, 10.4 Bodansky units; cholesterol, 150 mg.; cholesterol esters, 71 mg.
Hospital day of biopsy 1	1	2	2	2	3	2
Pathologic changes	Marked fat; focal necroses	Extreme fat	Extreme fat	Extreme fat; focal necroses; cirrhosis	Extreme fat	Extreme fat; focal necroses; cirrhosis
Hospital day of biopsy 2	8	9	9	9	10	9
Pathologic changes	Marked fat; cirrhosis	Marked fat	Marked fat	Marked fat; cirrhosis	Extreme fat	Extreme fat; focal necroses; cirrhosis
Hospital day of biopsy 3	15	16	16	16	17	16
Pathologic changes	Marked fat; cirrhosis	Marked fat	Moderate fat	Marked fat; cirrhosis	Marked fat	Extreme fat; focal necroses; cirrhosis
Hospital day choline treatment instituted	15	16	16	16	17	16
Gm. per day	3	3	3	6	9	9
Gm. total	42	42	42	84	126	126
Hospital day of biopsy 4	22	23	23	..	24	23
Pathologic changes	Marked fat; focal necroses; cirrhosis	Moderate fat	Moderate fat; cirrhosis	.....	Moderate fat	Marked fat; cirrhosis
Hospital day of biopsy 5	29	30	30	30	31	30
Pathologic changes	Marked fat	Marked fat	Moderate fat	Marked fat; focal necroses; cirrhosis	Slight fat; cirrhosis	Marked fat; cirrhosis

TABLE 2.—*Summary of Observations in Two Patients Treated with Choline*

Patient No.	7	8
Age, yr.	56	51
Sex	M	F
Race	W	W
Pertinent results of functional tests	Albumin, 3.4 Gm.; globulin, 3.8 Gm.; thymol turbidity, 6 units; alkaline phosphatase, 7.4 Bodansky units; cholesterol, 142 mg.; cholesterol esters, 99 mg.	Albumin, 4.7 Gm.; globulin, 4.0 Gm.; thymol turbidity, 12 units
Hospital day of biopsy 1	31	47
Pathologic changes	Marked fat; focal necroses	Moderate fat; focal necroses
Hospital day choline treatment instituted	34	53
Gm. per day	9	9
Gm. total	252	252
Hospital day of biopsy 2	41	60
Pathologic changes	Marked fat	Moderate fat; focal necroses
Hospital day of biopsy 3	48	67
Pathologic changes	Moderate fat; focal necroses	Slight fat; focal necroses; cirrhosis
Hospital day of final biopsy	62	81
Pathologic changes	Moderate fat; focal necroses; periportal tubercle	Slight fat; focal necroses

a change from marked fatty metamorphosis to normal liver occurred in the same time and on the same regimen as in the control period in patient 6.

It is unfortunate that biopsies were not obtained from patients 7 and 8 (table 2) at the time of their admission to the hospital. Fatty change was discovered in these patients on the thirty-first and forty-seventh hospital days, respectively, during the investigation of 70 patients with pulmonary tuberculosis for the presence of hepatic disease. These two periods were considered as controls comparable to the two week period used in the other six patients, since both these patients had been on a diet of 3,400 calories from the time of their admission. Fatty change was observed in 14 of these 70 patients. Of these, 11 were among the 32 patients whose biopsies were obtained within 18 days of admission. The remaining three were among the 38 patients whose biopsies were obtained between the eighteenth and three hundred and fifty-sixth hospital days. Patients 7 and 8 were selected from these three patients for treatment with choline because the degree of fatty change was greater than expected. While there was diminution in the amount of fat in the liver after one month of choline administration, it was not considered significant enough to conclude that choline was of particular value in accelerating the fat clearance in these persons.

SUMMARY AND CONCLUSIONS

The rate of fat clearance in a number of patients with fatty metamorphosis while on routine house diets is discussed. The effect of choline supplements on the rate of fat clearance in eight patients with fatty metamorphosis is presented. The majority of patients showed rapid reduction in fat content on diet alone. The administration of choline supplements did not accelerate the rate of fat clearance.

RIEHL'S MELANOSIS AND THE ADRENAL GLANDS

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A CASE OF Riehl's melanosis was attributed [1] to adrenal insufficiency resulting from the protracted ingestion of toxic amounts of fluorine which sensitized the skin to the action of the sun's rays. Treatment directed against chronic fluorine poisoning led to the disappearance of the facial pigmentation and of the coexistent patches of melanoderma on various parts of the body.

In the following paragraphs another case of Riehl's melanosis is recorded.

REPORT OF A CASE

A retired civil servant, aged 66, was from his boyhood subject to frequent attacks of a condition diagnosed as "influenza," which recurred three or four times a year. Laryngitis was a frequent complication, and an attack in 1946 left him with the loss of the senses of smell and taste. He never recovered the former, but the latter improved, though without ever returning to normal. The underlying cause of the hyperpyrexia was never discovered, and several blood examinations for malarial parasites gave negative results. In 1919, after an air crash, severe thrombosis developed in one leg. In 1923 there was an attack of scarlet fever and rheumatic fever, which resulted in the action of the heart on occasions becoming irregular.

For several years there was peeling of the skin between the toes, spreading to the feet, and a similar condition occurred on the fingers and hands. His nails were brittle, but his hair did not fall out to any extent. He had constipation and flatulence. When not affected by "influenza," he felt very fit both physically and mentally.

When I first saw him in 1937, I could find nothing to help elucidate the nature of the recurrent feverish attacks. In view of his digestive symptoms, however, large doses of charcoal and an aperient were recommended, so as to eliminate the possibility that the attacks might originate in the alimentary canal. I have never seen him during any of his attacks, but after a lapse of years he volunteered the information that they were not nearly so frequent or so severe as they were before the prescribed regimen was instituted.

He stated that he had had a dark complexion for many years, but, since I always saw him indoors, or during the hot season, either the bronzed appearance of his face escaped my attention altogether or it was so slight that I attributed it to ordinary tanning by the sun's rays. My attention to his present marked facial pigmentation

From the Department of Physiology, Middlesex Hospital Medical School.

1. Spira, L.: Acta dermat.-venereol. (*a*) **27**:83, 1946; (*b*) **29**:27, 1949.

was, however, attracted for the first time when I met him in the open during the summer of 1948. In 1919, after the air crash, he noticed a bare patch on each side of the chin. At a later stage, white strands of hair grew from these patches. In the course of the next few years, the hair at the temples and at the back of the head changed color four or five times from black to gray. After an exposure to the sun's rays, a brown discoloration developed on the face and neck. Some years later, the color of the scalp on the forehead, above the line where it is touched by the rim of the hat, changed to pink, thus sharply contrasting with the brown discoloration on the face and neck.

Clinical Findings.—The facial discoloration (fig. 1) was irregular in its distribution and consisted of light brown and dark brown patches which merged imper-

Fig. 1.—Appearance of patient with Riehl's melanosis.

ceptibly into one another. On the forehead, about 1 to 2 cm. below the hair margin, the brown color ceased abruptly, being separated by a serrated line of demarcation from an area which appeared grossly depigmented but was actually bright pink, comparable with the color produced on insolation of normal skin. White depigmented areas were seen to the left of, and underneath, the chin, along the nasolabial folds and, more particularly, on the deeply pigmented throat. The dorsa of both hands were also involved, the unequally distributed pigmentation stopping sharply at the metacarpophalangeal joints. The buccal mucous membrane was free from pigmentation. There was slight cheiropompholyx on some of the fingers, and dhobie itch was present in the interdigital spaces of the feet. His finger and toe nails exhibited longitudinal striation (onychorrhexis) and pronounced transverse furrows (Beau's lines).

His blood pressure was 120/60 mm.Hg, and both the hypopiesia and the presence of arrhythmia were indicative of chronic myocardial degeneration. The electrocardiogram was, however, normal (Dr. G. E. S. Ward, Middlesex Hospital), and nothing of note was found in any of the internal organs. His legs presented severe varicose veins.

To throw further light on the hypothesis [1] that Riehl's melanosis may be due to chronic adrenal insufficiency caused by a disturbance of the vegetative nervous system in the course of chronic fluorine poisoning (in which other endocrine glands, especially the parathyroids, may be simultaneously affected), a series of laboratory tests were carried out.

Laboratory Examination.—Results of biochemical tests (Dr. A. E. Kellie, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School) were as follows: The urine showed less than 0.005 Gm. of total protein per 100 ml. and no sugar. No hematoporphyrin was detected. Tests for 17-ketosteroids revealed excretion of 4.4 mg. of sterone per day. Blood urea measured 48 mg. per 100 ml.

Serum chlorides were 579 mg., potassium, 24.6 mg., sodium, 320 mg., phosphates, 4 mg., and calcium, 10.5 mg., per 100 ml. Alkaline phosphatase measured 10 units.

In the dextrose tolerance test (fig. 2) the resting specimen showed 75 mg. of sugar, that taken at one-half hour, 90 mg., that taken at one hour, 94 mg., that taken at 1½ hour, 76 mg., and that taken at two hours, 80 mg., per 100 ml. The following tabulation lists the results of urinalysis during the tolerance test.

	Volume, Ml.	Specific Gravity	Sugar	Rothera's Test for Acetone	Gerhardt's Test for Acetoacetic Acid
Rest	115	1.017	0	Negative	Negative
1 hr.	85	1.015	0	Negative	Negative
2 hr.	90	1.014	0	Negative	Negative

No protein or sugar was detected in the resting urine specimen.

Resting gastric juice obtained after a fractional test meal had a normal odor and showed absence of bile, blood and mucus. Later specimens also showed absence of bile and mucus.

Further examination of the blood revealed the following values (Bland-Sutton Institute of Pathology, Middlesex Hospital). The Wassermann reaction was negative; the erythrocyte sedimentation rate was 8 mm. per hour. There were 5,000,000 erythrocytes per cubic millimeter. The hemoglobin concentration was 103 per cent. The color index was 1.03. Leukocytes numbered 7,800 per cubic millimeter, with 64 per cent neutrophils, 29 per cent lymphocytes and 7 per cent monocytes.

Histologic Observations.—Biopsy (figs. 4 and 5) of a section of skin from the forehead, which included areas of both pigmentation and depigmentation, gave the following results (Dr. A. C. Thackray, Bland-Sutton Institute of Pathology, Middlesex Hospital). The section showed a strip of skin in which there was some general thinning of the epidermis, with hyperkeratosis of the mouths of the follicles. At one end of the section there was greatly increased pigmentation of the epithelium, and in this region there were groups of pigment-laden chromatophores in the underlying dermis. In addition, collections of chronic inflammatory cells, mostly lymphocytes, were seen in the superficial part of the dermis. In sections stained by Fontana's method, the pigment appeared as dense black granules; the granules were unaffected in sections stained to demonstrate free iron. The appearances were those of Riehl's melanosis.

Results of Roentgenographic Examination (Sir Harold K. Graham Hodgson, Radiological Department, Middlesex Hospital).—No evidence of calcification was

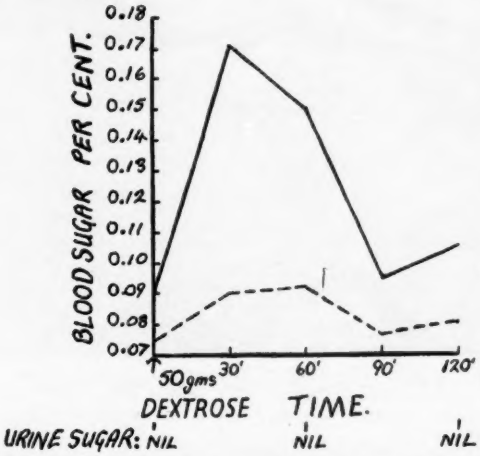

Fig. 2.—Graph showing results of dextrose tolerance test. Average normal values are indicated by solid line and values for patient by broken line.

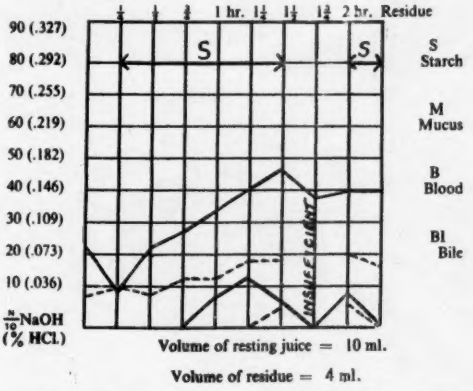

Fig. 3.—Graph showing results of fractional test meal. Normal values are indicated by solid line and values for patient by broken line.

noted in the adrenal glands. The pituitary fossa was normal. There was no evidence of osteoporosis of the bones of the forearms or hands.

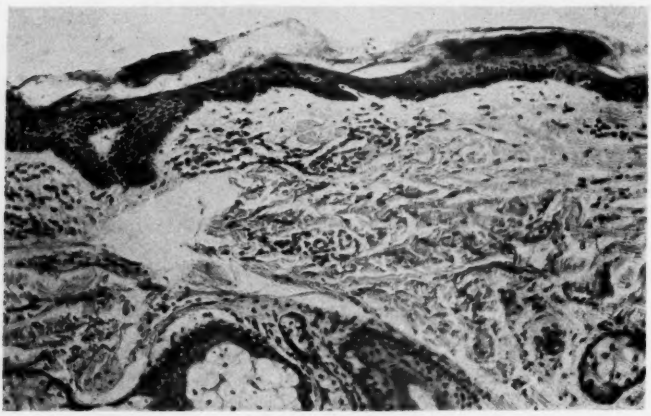

Fig. 4.—Photomicrograph of section of skin from forehead; × 109.

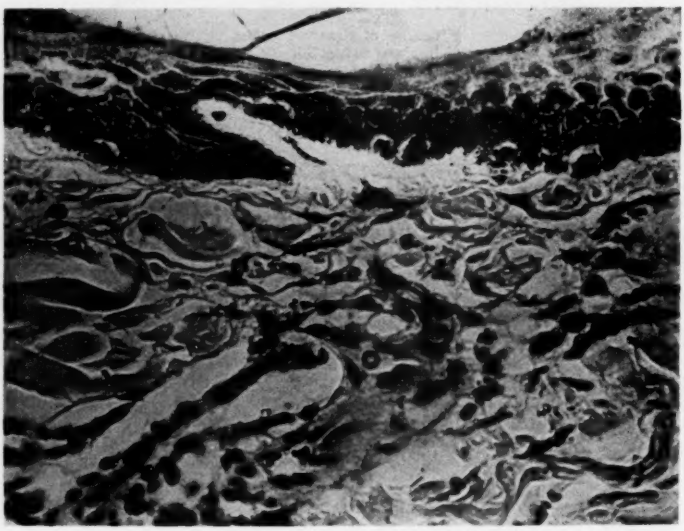

Fig. 5.—Photomicrograph of section of skin from forehead; × 520.

Examinations for Fluorine and Arsenic.—The endemic nature of fluorosis has been traced [2] to the protracted ingestion of toxic amounts of fluorine contained in variable quantities in practically every article of food and drink. Since it is the drinking water, however, which is usually incriminated as the main source of the noxa, the tap water from the patient's residence was submitted for chemical analysis. Because of the similarity in the actions of fluorine and arsenic, the analysis was extended to include both these noxae. The following is the result of the analysis (Mr. D. D. Moir, Public Analyst for the Borough of Malden and Coombe, Surrey). The sample of tap water contained no arsenic expressed as arsenic trioxide (As_2O_3) and 0.15 part per million of fluorine.

According to Spira,[2] hair and finger and toe nails are important channels through which fluorine, like arsenic, is eliminated from the body. Samples of the patient's hair and nails were submitted for analysis. The Public Analyst reported that the sample of hair, after washing, contained 0.4 part per million of arsenic expressed as As_2O_3 and 2 parts per million of fluorine. The sample of finger and toe nails, after washing, contained 5 parts per million of arsenic expressed as As_2O_3 and 30 parts per million of fluorine. The arsenic content of the nails and hair samples was, if anything, lower than average. As far as I know, there is no reliable information on the fluorine content of nails and hair. The methods used were as follows: For detection of arsenic wet destruction of organic matter was carried out, followed by reduction of arsenic to the trivalent condition and determination by the Gutzeit method. The method used for detection of fluorine was based on that of the report of the Analytical Methods Committee of the Society of Public Analysts.[3] The nails required a preliminary distillation from perchloric acid and the hair from 1:1 sulfuric acid. In the latter case steps were taken to exclude interference by sulfides or sulfites.

COMMENT

Hyperpigmentation, hypopiesia, hypochlorhydria and hypoglycemia are all cardinal signs of chronic adrenal insufficiency. The frequent occurrence of influenza, and the diminished excretion of 17-ketosteroids which is indicative of adrenal cortex insufficiency, are additional evidence that in the case of Riehl's melanosis here reported the adrenal glands were vitally involved. A disturbance of the electrolyte balance plays an essential part in Addison's disease. Although in the present case the concentration of serum sodium and serum chlorides was practically normal, the potassium content of the blood serum was significantly increased, amounting to 24.6 mg. per 100 ml., compared with 17 to 22 mg. per 100 ml. in a normal subject. In spite of all these findings, in the absence of other clinical signs and symptoms, such as asthenia, more generalized pigmentation involving also the buccal mucosa, pulmonary disease and calcification of the adrenal glands, a diagnosis of Addison's disease would, however, not be justified. The patient's general health was not impaired, and, apart from his recurrent attacks of influenza, he had no complaints.

2. Spira, L.: Acta med. Scandinav. **130**:78, 1948.

3. Determination of Fluorine in Foods: Report of a Sub-Committee of the Analytical Methods Committee, Analyst **69**:243, 1944.

It will, on the other hand, be remembered that Garnier [4] recorded the case of a woman suffering from Riehl's melanosis who died a year later of Addison's disease.

The high level of blood urea (48 mg. per 100 ml.) found in the present case may have an important significance. Grollman [5] emphasized the retention of nonprotein nitrogen in adrenal insufficiency and cited the work of Marshall and Davis,[6] who demonstrated the rise in the nonprotein nitrogen (particularly the urea) concentration of the blood which follows adrenalectomy in cats. Rowntree [7] observed an increase in the blood urea in patients with Addison's disease which is proportionate to the degree of adrenal insufficiency. It is suggested that there may be some relationship between this fact and the statement of Waddell [8] that urea elimination increases under the influence of potassium fluoride, a substance which does not act as a mere eliminator of urea but actually increases the formation of urea through excessive destruction of tissue.

In his original report Riehl [9] described the pigmentation as being similar to that encountered in chronic arsenical poisoning or in pellagra. In the present case against the diagnosis of chronic arsenical poisoning is adduced the low excretion of the noxa in the hair and nails. Nor is the absence of a history of a stringent dietary restriction (apart from that inherent in the present postwar period), of diarrhea and of mental symptoms, such as occur in pellagra, compatible with a diagnosis of this disease, even though the pigmentation of the hands described is identical with the striking changes in the hands and wrists of patients suffering from pellagra.

The diagnosis of vitiligo was considered on the basis of the prominent areas of depigmentation on the face, neck and hands. Vitiligo is characterized by the occurrence of milk-white patches, usually presenting hyperpigmented borders and surrounded by normal or increased pigmentation. In the present case the increased pigmentation was a predominant feature from the outset, the light areas supervening at a later stage. It has been suggested that vitiligo is a trophoneurosis belonging to the same category as alopecia areata; in the opinion of others it is caused by an unknown toxin.

4. Garnier, G.: Bull. Soc. franç. dermat. et syph. **45**:900, 1938; Nature, London **156**:163, 1945.

5. Grollman, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

6. Marshall, E. K., and Davis, D. M.: J. Pharmacol. & Exper. Therap. **8**:525, 1916; cited by Grollman.[5]

7. Rowntree, L. G.: Studies in Addison's Disease, J. A. M. A. **84**:327 (Jan. 31) 1925.

8. Waddell, L. A.: J. Anat. & Physiol. **18**:145, 1884.

9. Riehl, G.: Wien. klin. Wchnschr. **30**:780, 1917.

It is submitted that in the present case the occurrence of small patches of alopecia areata on the chin, which preceded the development of the pigmentary changes, was one of the manifestations of the coexistent hypoparathyroidism, its other signs being brittleness of the nails, onychorrhexis and Beau's lines. According to Spira,[10] any fungus that may be found in certain cases of cheiropompholyx and dhobie itch in the course of chronic fluorine poisoning should be looked on as a secondary infective agent. The frequent occurrence of varicose veins has been shown [11] to be due to loss of elasticity of the vascular system, resulting from the calcium-precipitating action of fluorine.

According to Spira,[12] in fluorosis signs and symptoms attributable to various endocrine glands are brought about by a disturbance in the coordination of those fibers of the sympathetic and parasympathetic nervous systems which supply the respective glands, and it will depend on the degree and the extent of their dysfunction which of the various syndromes will prevail over the others. Thus, in Riehl's melanosis (as indeed in any other pigmentary disturbance) the dysfunction of the chromaffin system governs the clinical picture; in hypoparathyroidism the ectodermal dystrophies preponderate. In either case symptoms attributable to the other condition remain in the background.

In the case here recorded, the parathyroids appeared to be only slightly involved; the serum calcium, phosphates and phosphatase were all within normal limits, although clinically the ungual changes indicated a calcium deficiency of the body. In fact, the disturbance of the vegetative nervous system has been ascribed to the calcium content being diminished by the specific action of fluorine.

An endeavor was made to improve the patient's condition by the method which proved successful in another case of Riehl's melanosis.[1b] Large doses of charcoal and an aperient were recommended to adsorb and eliminate the noxa accumulated in the body, and adrenal extract in doses of 0.15 Gm. (2½ grains) was prescribed to be taken twice a day to supplement the adrenal deficiency. To replenish any lack of calcium (indicated by the presence of ectodermal dystrophies), calcium lactate was given in massive doses, and the further ingestion of fluorine, as contained in large quantities in tea, among other sources, was prohibited. Owing to prevailing circumstances, this regimen could not be strictly carried out, particularly since it involved restrictions not easily accepted by anyone who, apart from the facial discoloration, was in perfectly good health. In spite of these difficulties, the patient reported that there was a definite improvement in the eruption on his hands and

10. Spira, L.: J. Hyg. **45**:93, 1947.

11. Spira, L.: Med. Press **219**:106, 1948.

12. Spira, L.: Acta med. Scandinav. **126**:65, 1946.

feet. On the fingers the spots which used to expand and involve areas of loose skin disappeared altogether. Somewhat later, the same improvement occurred also in the toes. The sharp line of demarcation between the deeply pigmented face and the depigmented forehead became blurred, and there was an obvious change taking place. Unfortunately, another attack of influenza with a temperature of 105 F. and complicating pneumonia developed. The treatment against fluorosis was stopped and for external reasons not resumed after recovery.

The exact cause of vitiligo is unknown and that of pellagra by no means certain. Further investigation will be needed to determine whether both these conditions are not to be considered as belonging to the common group of pigmentary disturbances caused by fluorine and, like Riehl's melanosis, responding favorably to elimination of fluorine in the same way as the pigmentation in chronic arsenical poisoning reacts promptly to the excretion of arsenic from the body.

SUMMARY

A case of Riehl's melanosis is recorded, in which hypopiesia, hypochlorhydria, hypoglycemia, diminished excretion of 17-ketosteroids, high blood urea content and frequent attacks of "influenza" indicated that the adrenal glands were vitally involved.

Mr. R. C. Syms, St. John's Hospital for Diseases of the Skin, performed the biopsy, and Mr. E. C. Claydon, Bland-Sutton Institute of Pathology, Middlesex Hospital, prepared the microscopic slides. Mr. E. V. Willmott, British Postgraduate Medical School, took the photomicrographs and Mr. Maurice Turney, Middlesex Hospital, all the other photographs.

HEMATOLOGY OF ATOMIC BOMB CASUALTIES

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CHICAGO

As a practical generalization, it may be said that sufficient irradiation of tissues with either radium or roentgen rays will cause a reduction in the number of cells of all series in the circulating blood. The greater the dose, the more profound is the blood damage, the more rapidly it develops and the more slowly it is repaired. The blood response remains quite consistent qualitatively over a wide range of dosage, although massive exposures may obscure the early changes, and small doses sometimes fail to evoke the complete response.—Minot and Spurling.[1]

DURING the period Aug. 6 to Dec. 16, 1945, a large number of hematological studies was performed on Japanese injured by the atomic bombs exploded over Hiroshima and Nagasaki, Japan. These studies were conducted by Japanese physicians and by medical officers of the United States Army and Navy attached to the Joint Commission for the Investigation of the Effects of the Atomic Bomb in Japan. The results of the studies were submitted in the Medical Report of the Joint Commission [2] and have not been published in their entirety up to the present. Because of current interest in the syndrome of radiation injury from the standpoint of national defense, permission has been granted for the publication of these detailed hematological studies. The source of the material here presented is Section 6, hematological aspects, and Section 9, statistical analysis, of the Medical Report. The data are of unusual interest as the authoritative record of the changes in the blood of a large group of persons exposed to varying amounts of ionizing radiation from atomic bombs. They are of especial interest when com-

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This paper has been reviewed in the Office of the Director of Classification, United States Atomic Energy Commission, and contains no restricted data as defined by the Atomic Energy Act of 1946.

1. Minot, G. R., and Spurling, R. G.: The Effect on the Blood of Irradiation, Especially Short Wave Length Roentgen Ray Therapy, Am. J. M. Sc. **168**:215-241, 1924.

2. Medical Report of the Joint Commission for the Investigation of the Effects of the Atomic Bomb in Japan, Atomic Energy Commission Classified Document, 1946.

pared with the report of Snell,[3] of the United States Atomic Bomb Casualty Commission. In the preparation of the latter report many of the Japanese studied were the same persons discussed in the present paper.

A large body of literature is available describing the hematological changes that occur in laboratory animals after exposure to ionizing radiation, and it is not necessary to review it at this time. The interested reader is referred to the monographs of Dunlap[4] and Bloom.[5] This report will be restricted to a description of changes that were observed in the peripheral blood. An interpretation of the changes in the bone marrow is in preparation. The clinical features of radiation injury were discussed by Warren and Bowers.[6]

MATERIAL AND METHODS

The material on which this study is based was obtained from three sources: 1. Blood examinations performed by Japanese physicians prior to the formation of the Joint Commission (about Sept. 15, 1945). Some of these studies were made by medical aid and research groups recruited from various medical schools. Others were carried out in the wards of medical schools and military hospitals to which casualties had been admitted. Few of such studies were systematic, and most were done under extremely unfavorable conditions. 2. Routine blood counts performed on survivors in each city as a part of the program of the Joint Commission. An attempt was made to obtain a reliable "sample" of persons from all parts of the bombed cities. The study included the completion of a questionnaire, a physical examination and a routine blood count by American and/or Japanese technicians. 3. Special hematological studies conducted by members of the Joint Commission in the casualty hospitals to which they had access.

The tabular data which follow will be identified as follows: *JAP*, Japanese data, and *JC*, data from the Joint Commission statistical study. In most instances the material from the Medical Report[2] has been revised to facilitate presentation, and new graphs have been prepared.

The laboratory methods employed were as follows:

Erythrocytes and leukocytes were counted with standard hemacytometers. Some of these were of American manufacture, and the others were of Japanese or German make. None of the equipment was certified by the National Bureau of Standards. When time permitted, counts were made in duplicate, but these were an exception.

The hemoglobin concentration was estimated with the Haden-Hausser or the Sahli hemoglobinometer (American and Japanese models) and by the copper

3. Snell, F. M.; Neel, J. V., and Ishibashi, K.: Hematologic Studies in Hiroshima and a Control City 2 Years After the Atomic Bombing, Arch. Int. Med. **84**:569-604 (Oct.) 1949.

4. Dunlap, C. E., III: Effects of Radiation on the Blood and the Hematopoietic Tissues, Including the Spleen, the Thymus and the Lymph Nodes, in Warren, S.: Effects of Radiation on Normal Tissues, Arch. Path. **34**:562-608 (Sept.) 1942.

5. Bloom, W.: Histopathology of Irradiation from External and Internal Sources, National Nuclear Energy Series IV, New York, McGraw-Hill Book Company, Inc., 1948.

6. Warren, S., and Bowers, J. T.: The Acute Radiation Syndrome in Man, Ann. Int. Med. **32**:207-216, 1950.

sulfate ($CuSO_4$) method. The hemoglobinometers were standardized against the $CuSO_4$ method, and, for the Japanese, 14.5 Gm. of hemoglobin per 100 ml. was taken as 100 per cent. The standard $CuSO_4$ solutions were checked by the pyknometer method. The hematocrit determinations (for mean cell volume, etc.) were done by Wintrobe's method.

Differential counts of 50 to 200 cells were made from films prepared on slides or cover slips stained with Wright's stain. Most of these films are now filed at the Armed Forces Institute of Pathology

Thrombocyte (platelet) counts and clotting studies were performed by a variety of methods and, generally speaking, were not reliable.

The exigencies of the situation were such that the usual niceties of clinical research could not always be observed. In spite of this, there was remarkably good agreement between the findings obtained by various groups in each city.

SELECTION OF DATA

The large number of blood counts available for analysis and the variation in the amount of radiation received by persons throughout the bombed cities forced me carefully to select the data for presentation. The questionnaire used in the Joint Commission study yielded information on the approximate location of each person with respect to zero (the point on the ground directly beneath the bomb), his position (i. e., outdoors or inside a building), his clinical symptoms, the extent of his injuries, if any, and the results of physical examination and blood examination. The material on the questionnaires was transcribed to International Business Machine (IBM) cards for statistical analysis.[7] Of the 13,545 questionnaires suitable for analysis, approximately one half contained some hematological data. In selecting material to demonstrate the effect of atomic bomb radiations on the blood picture, two groups of persons were used: (1) patients in whom the diagnosis of "radiation injury" was made and (2) persons exposed to comparable amounts of ionizing radiation who survived 20 days or more. It is desirable to define these two groups precisely, so that the reader may appreciate both the significance and the limitations of the hematological studies of atomic bomb casualties.

1. *Radiation Injury.*—One of the objectives of the Joint Commission was to establish criteria for the diagnosis of radiation injury in men. From the 14 clinical signs and symptoms recorded on the questionnaires it was possible by analysis to define two as specific symptoms of radiation injury, epilation and purpura, and three as suggestive symptoms, vomiting on the day of the bombing, oropharyngeal lesions (such as pain, ulceration, necrosis and gingivitis, occurring within 39 days after exposure) and hemorrhagic manifestations other than purpura. The correlation of these criteria with the blood changes and with the estimated dose of radiation was excellent. In the tables that follow, "persons with radiation injury" have been selected on the basis of the foregoing specific and/or suggestive symptoms. The interrelationship between the various clinical types of radiation injury is shown in table 1. It should be noted that all the persons classed as having radiation injury had been or were obviously ill at the time they were studied.

2. *Persons Exposed to Comparable Amounts of Radiation.*—In the evaluation of the Japanese casualties, it was desirable to divide the patients into groups, as homogeneous as possible, in respect to their probable exposure to radiation. If the exact amount of shielding material between each person and the bomb had been known, as well as the exact distance, it would have been theoretically possible to

7. The statistical analysis was carried out under the supervision of Major E. Cuyler Hammond, Air Corps, in the Office of The Air Surgeon.

make precise classifications. While such a procedure could not be applied, it was practical to make reasonably homogeneous groupings from the information available on the influence of shielding (by heavy building construction, shelters, etc.) on the incidence of symptoms. Within the same distance, persons outdoors or in wooden buildings were most exposed to radiation, persons in heavy-type buildings were less exposed and persons in bomb shelters and tunnels were least exposed. It

TABLE 1.—*Classification of Clinical Types of the Syndrome of Radiation Injury**

Pathological group †	I	II	III	IV
Clinical designation	Very Severe	Severe	Moderately Severe	Mild
Approximate mortality, %	100	50	10	Nil
Time of death, wk	1 to 2	3 to 6	6 +	Nil
Exposure group	A	A, B	C, D	D, E, F, G
Approximate distance, yd. from zero, for persons outdoors, unshielded or in Japanese buildings	1,100	1,100 to 1,650	1,650 to 2,750	2,200 +
Symptoms and Signs				
Vomiting, day of bombing ‡	+	+	+	+
Epilation	±	+++	+	±
Purpura	+	+++	++	+
Leukopenia	+++	+++	++	+
Other suggestive signs of radiation injury	+	+++	++	+

* Source: table 1, section 6, Medical Report.[2]
† These designations are the ones used by Liebow, Warren and deCoursey (Am. J. Path. 25: 853-1027, 1949).
‡ Vomiting on the day of bombing is placed in the first rank of symptoms because of its obvious usefulness in triage and early evaluation of individual patients.

TABLE 2.—*Comparison of Exposure Groups According to Distance from Zero, Yards**

Exposure Group	Persons Outdoors or in Wooden Buildings	Persons in Heavy Buildings	Persons in Bomb Shelters and Tunnels
A	0 to 1,100 †	.....	.....
B	1,100 to 1,650	0 to 1,100	.....
C	1,650 to 2,200	1,100 to 1,650	0 to 1,100
D	2,200 to 2,750	1,650 to 2,200	1,100 to 1,650
E	2,750 to 3,300	2,200 to 2,750	1,650 to 2,200
F	3,300 to 4,400	2,750 to 3,300	2,200 to 2,750
G	4,400 to 5,500	3,300 to 5,500	2,750 to 5,500
H	Beyond 5,500	.....	.....

* Source: page 50, section 9, Medical Report.[2]
† For simplicity of calculation, 1,100 yards is considered equal to 1,000 meters.

was observed that, roughly, the incidence of the more important symptoms among persons in heavy buildings was about the same as the incidence of the same symptoms in persons outdoors who were about 550 yards (500 meters) farther from zero. On this basis, eight exposure groups, lettered *A* to *H*, were made up by collating the IBM cards. The location of the persons in these exposure groups is shown in table 2.

Exposure group A consisted of the most exposed patients, and exposure group H, presumably of the least exposed. It is assumed that all persons in each exposure

group received about the same average dose of radiation. It is apparent that within any exposure group the persons who did not manifest radiation injury (as defined) either had greater resistance or were better shielded.

In the data labeled *JAP*, there has been a considerable, but unknown, amount of selection. Blood counts were made only on the critically ill or on patients who presented the syndrome which the Japanese came to recognize as radiation injury. It is fair to assume that patients so designated had radiation injury in most instances. Only those reports concerning the validity of which I have no doubt are included.

In the data labeled *JC*, there has been no selection in the usual sense of the term. The data are presented in relation to distance, to exposure groups, to other injuries and to the time trend. Because of the manner in which the data were collected, relatively few of the persons had serial blood examinations. For this reason, it was necessary and proper to deal with pooled blood counts to demonstrate the time trend of the changes in the blood picture. All the blood counts recorded on the questionnaires for which a date was noted are included in the tabulations. Differential counts were excluded if the percentages added up to less than 95 or more than 105. All the blood count data are presented in terms of weeks after the bombing. In the Medical Report [2] week 1 is defined as the first seven days following the day of the bombing, week 2 the next seven days and so on. The data have been combined in various ways (i. e., weeks 2 to 5; exposure groups *A* and *B*, etc.) to obtain large enough numbers of counts for analysis, to demonstrate significant trends, etc. The number of white blood cell counts available for analysis is shown in table 3.

TABLE 3.—*Summary of Data**

Exposure Group	Hiroshima		Nagasaki	
	No. of Persons	No. of White Cell Counts †	No. of Persons	No. of White Cell Counts
A	570	387	376	161
B	1,119	670	1,465	592
C	1,817	965	1,630	554
D	1,604	794	702	156
E	711	286	663	132
F	575	145	1,091	157
G	267	77	500	70
Total	6,663	3,314	6,427	1,822
H	219	112	194	94
Grand Total	6,882	3,426	6,621	1,916

* Source: tables 23H, 23N, 33H and 33N, section 9, Medical Report.[2]
† These are single blood counts on different persons, living 20 days after the bombing, who were examined at any time between weeks 1 and 12 inclusive.

OBSERVATIONS

The fact that radiation injury of varying degrees of severity occurred complicates the presentation of the hematological findings. It is desirable, however, to present the apparently typical blood pictures for the two types of the syndrome which were the most serious, very severe and severe radiation injury. Although it is not strictly correct to do so, it is probably a fair generalization to assume that most of the survivors who were included in the Joint Commission study had sustained radiation

injury which could be graded as moderately severe or mild. Actually, it is not possible to use the data from the questionnaire study in a clinical sense, since the severity of radiation injury cannot be determined for groups of persons. With reference to table 1, however, it is reasonable to assume that most of the persons in exposure groups A and B sustained severe radiation injury if they survived 20 days or more, and that the persons in exposure groups C and D and E, F and G sustained moderately severe and mild radiation injury, respectively. However, this assumption is less valid than the clinical evidence.

First the Japanese data reported for patients who are certainly identifiable as examples of very severe and severe radiation injury are considered. It may be seen in figure 1 that the time trend of pooled white blood cell counts for exposure groups A and B correspond closely with

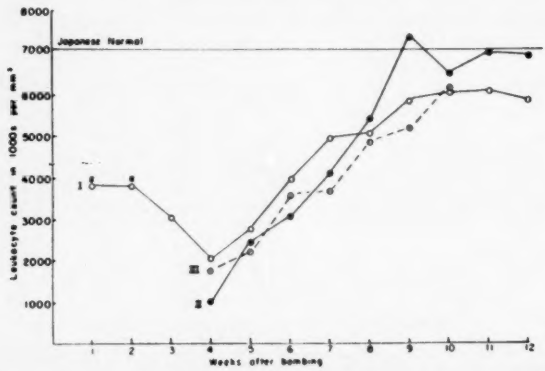

Fig. 1.—Time trend of leukocyte count in severe radiation injury. Curve I shows data for exposure groups A and B, pooled (1,057 persons); curve II, for exposure groups A and B, pooled, of 287 persons with specific signs of radiation injury, and curve III, for 16 patients with severe radiation injury from Misao Clinic. Stars indicate average of less than 10 counts.

serial counts for patients in well run hospitals who had severe radiation injury. The similarity of these curves is a reasonable basis for accepting the clinical conclusions of reliable Japanese physicians with respect to severe radiation injury. As a matter of interest, it should be noted that the median lethal dose in 90 days of ionizing radiation for man was delivered at about 1,100 yards (1,000 meters).

VERY SEVERE RADIATION INJURY

All the hemograms of patients with very severe radiation injury were obtained from the Japanese. Because of the chaotic conditions in each city, few valid studies are available. All the reliable data that were

obtained by the Joint Commission are shown in tables 4 and 5. Of the very severe cases from Hiroshima records were available of nine differential counts. These are reproduced in table 6. It is apparent that there was no characteristic hemogram at this time, since the proportion of the various cell types is about normal. Other observers reported that only

TABLE 4.—*Blood Counts of Patients with Very Severe Radiation Injury: Hiroshima Data (JAP)**

Case No.†	Days After Bombing: Death	Days After Bombing: Count	White Blood Cells	Hemoglobin Concentration, %	Red Blood Cells, Millions ‡
H-10608-I	6	4	250	..	.....
.....	6	5	400	..	.....
H-10604-I	7	5	1,440	..	.....
H-10634-I	6	6	120	..	.....
H-10612-I	6	6	560	..	.....
H-10606-I	8	6	840	..	.....
H-10611-I	8	6	3,400	..	.....
H-10620-I	8	6	2,100	..	.....
H-10618-I	8	6	2,000	..	.....
H-10629-I	8	6	150	..	.....
H-10643-I	9	6	920	..	.....
H-10636-I	10	6	300	..	.....
.....	..	7	25	..	.....
H-10609-I	9	7	50	..	.....
H-10640-I	9	7	560	..	.....
.....	..	8	740	..	.....
.....	..	8	37	..	.....
.....	..	8	490	..	.....
.....	..	8	80	..	.....
H-10626-I	9	8	100	..	.....
.....	..	9	200	..	.....
.....	..	9	550	..	.....
.....	10	9	200	..	.....
H-10614-I §	9	6	400	42	4.10 B W
H-10619-I §	9	6	150	50	4.20 B W
H-10645-I §	11	6	400	45	4.20 B
H-10615-I §	8	6	400	32	2.65 B
H-10616-I §	8	6	150	43	3.28 B
..... §	..	6	250	40	3.75 B W
H-10635-I §	8	6	300	40	2.82 W
H-10625-I §	8	7	400	40	4.13 B
..... §	9	7	300	40	3.26 W

* Source: tables 15 and 16, section 6, Medical Report.[2]
† Case numbers refer to Joint Commission records system.
‡ B indicates burns and W wounds.
§ This group of patients was inside a heavy concrete building, 220 yards (200 meters) from zero.

lymphocytes were seen in the blood films made during the first week, but no films or reliable records of such films were located. The structure of the few cells that remained appeared normal except that some of the neutrophils were "giant polymorphonuclear cells."

The blood counts of these patients are of interest because of the rapidity with which leukopenia occurred in human beings exposed to

amounts of radiation considerably in excess of the estimated median lethal dose.

TABLE 5.—*Leukocyte Counts of Patients with Very Severe Radiation Injury: Nagasaki Data (JAP)**

Case No.	Days After Bombing: Death	Days After Bombing: Count	White Blood Cells
4739	2	1	2,200
4733	2	1	1,670
.....	..	2	200 to 530
.....	..	2	830
4737	2	2	1,300 to 1,600
4738	2	2	600 to 1,000
4736	2	2	400 to 500
4740	2	2	530 to 1,040
4734	2	2	830
4732	2	2	300
4731	2	2	715
4793	8	8	430 to 500
4799	9	9	1,700
4795	9	9	500

* Source: table 14, section 6, Medical Report.[2]

TABLE 6.—*Differential Counts on Patients with Very Severe Radiation Injury (JAP)**

Case No.	No. Cells Counted	Per Cent of Nucleated Cells: Metamyelocytes	Stab Cells	Polymorphonuclear Cells	Lymphocytes	Monocytes	Eosinophils	Plasma Cells	Nucleated Red Blood Cells
H-10614-I	50†	0	20	40	28	6	0	2	0
H-10619-I	50	0	8	50	30	4	0	0	4
H-10645-I	50	0	16	60	14	10	0	0	0
H-10615-I	20	0	0	60	5	0	0	0	35
H-10616-I	13	0	15	85	0	0	0	0	0
H-10625-I	30	0	4	73	16	7	0	0	0
H-10635-I	20	0	0	90	5	5	0	0	0
.....	50	0	10	70	16	2	0	0	0
.....	100	4	26	56	8	4	2	0	0
Average	..	0.5	11.0	65.0	13.5	3.0	..	..	8.0

* Source: table 20, section 6, Medical Report.[2]
† In general, the number of cells counted was all that could be found.

SEVERE RADIATION INJURY

A great many blood examinations were made on patients with severe radiation injury. The data have been selected on the basis of the diagnoses of the Japanese attending physicians and of the exposure group in which the subjects were placed. The distribution of leukocyte counts in these patients is shown in tables 7 and 8.

There is no simple explanation for the discrepancy in the distribution of leukocyte counts of the persons included in the statistical study of the Joint Commission, as shown in table 8. When groups of patients with apparently similar diagnoses are compared, there is much less difference between persons from the two cities. The typical character of the changes in white cell blood count in severe radiation injury is better demon-

TABLE 7.—*Distribution of Lowest Leukocyte Counts in Patients with Severe Radiation Injury, Weeks 3 to 5 (JAP)**

White Blood Cell Count	Fatal Cases	Nonfatal Cases
0 to 500	14	1
501 to 1,000	7	8
1,001 to 2,000	4	10
Over 2,000	0	4
Total	25	23

* Source: table 16, section 6, Medical Report.[2]

TABLE 8.—*Distribution of Leukocyte Counts During Weeks 2 to 5 in Exposure Groups A and B (JC): Persons* Who Were Living Twenty Days After Bombing†*

White Blood Cell Count	Hiroshima		Nagasaki	
	A	B	A	B
0 to 499	22	15	2	7
500 to 999	37	27	12	21
1,000 to 1,999	51	41	15	28
2,000 to 2,999	36	38	11	35
3,000 to 3,999	23	35	11	23
4,000 to 4,999	16	22	4	23
5,000 to 5,999	4	12	4	21
6,000 to 6,999	2	10	7	9
Over 7,000	4	11	5	15
Mean	2,096	2,979	3,170	3,856
Pooled groups A and B	2,555		3,673	

* These are single counts for different persons.
† Source: tables 34H and 34N, section 9, Medical Report.[2]

strated when one considers the time trend of the mean leukocyte count. This is shown for three groups of persons in figure 1. Curve I is the weekly pooled leukocyte counts of 1,057 persons in exposure groups A and B from Hiroshima (source: figure 13 H and table 33 H, volume 9, Medical Report [2]). Curve II is the weekly pooled leukocyte counts of 287 persons with specific signs of radiation injury in exposure groups A and B from Hiroshima (source: table 21, volume 3, section 6, Medical Report [2]). Curve III is the weekly average leukocyte counts of 16 patients with severe radiation injury who were studied in the Misao

clinic, Kyushu Imperial University (source: table 19, volume 3, section 6, Medical Report [2]). The similarity of these three time trend curves is remarkable when one considers the origins of the data.

In all the blood examinations made on patients with the severe type of radiation injury, leukopenia was most prominent in weeks 3 to 5, and the return to normal values was seen in weeks 8 to 10. The typical time trend of the white cell count is also seen when pairs of counts on the same persons are compared. This was possible with some persons from

TABLE 9.—*Time Trend for Pairs of Leukocyte Counts, Exposure Groups A and B: Hiroshima (JC)* *

No. of Cases	First White Blood Cell Count		Repeat White Blood Cell Count	
	Week	Mean	Week	Mean
29	2 to 4	2,986	5 to 7	3,497
40	2 to 4	2,390	8 to 10	7,395
32	2 to 4	2,487	11 to 13	6,781
56	5 to 6	2,920	7 to 8	5,861
29	5 to 6	2,834	9 to 10	6,403
53	5 to 6	2,700	11 to 12	6,625

* Source: tables 36H and 36N, section 9, Medical Report.[2]

TABLE 10.—*Time Trend for Pairs of Leukocyte Counts, Exposure Groups A and B and C and D: Nagasaki (JC)**

Group	No. of Cases	First White Blood Cell Count		Repeat White Blood Cell Count	
		Week	Mean	Week	Mean
A and B	57	2 to 4	3,009	5 to 16	7,177
A and B	57	5 to 6	3,461	7 to 16	7,614
C and D †	54	2 to 4	3,907	5 to 16	6,531
C and D	47	5 to 6	4,194	7 to 16	7,413

* Source: tables 36H and 36N, section 9, Medical Report.[2]
† Time trend of leukocyte count in exposure groups C and D is shown for comparison.

exposure groups A and B. These comparisons are shown in tables 9 and 10.

The relationship between the tempo of the evolution of the syndrome of radiation injury and the severity has been discussed in another report.[8] It is important to note that the mean day of onset of epilation and of purpura (specific symptoms of radiation injury) was the twentieth and twenty-fifth day, respectively (source: table 18, section 9, Medical Report [2]), for persons in exposure groups A and B. It is quite evident that weeks 3 to 5 are the critical ones for casualties with severe radiation injury.

8. LeRoy, G. V.: The Medical Sequelae of the Atomic Bomb Explosion, J. A. M. A. **134**:1143-1148 (Aug. 2) 1947.

TABLE 11.—*Exposure Groups A and B with Specific Signs of Radiation Injury, Pooled Differential Counts by Weeks (JC)**

Week	No.	Average White Blood Cell Count	Polymorphonuclear Cells		Lymphocytes		Monocytes		Eosinophils		Basophils	
			No.	%	No.	%	No.	%	No.	%	No.	%
4	11	1,000	365	36.5	575	57.5	55	5.5	10	1.0	1	0.1
5	39	2,435	1,347	55.3	883	37.3	104	4.4	30	1.2	5	0.2
6	18	3,094	1,836	59.3	1,215	39.5	107	3.5	100	3.1	14	0.4
7	22	4,113	2,448	59.5	1,152	28.0	364	8.9	122	3.0	16	0.4
8	13	5,384	3,220	59.8	1,658	31.0	276	5.2	124	2.3	20	0.3
9	10	7,320	4,692	64.1	2,012	27.1	557	7.5	236	3.2	..	...
10	17	6,576	3,922	59.6	1,940	29.7	331	5.1	292	4.5	37	0.5
11	66	7,048	4,248	60.3	2,048	31.0	343	4.9	340	4.8	25	0.4
12	91	6,908	4,031	58.4	2,080	30.1	369	5.3	390	5.7	25	0.3
†	28	7,042	3,745	53.2	2,660	38.0	350	5.0	252	3.6	35	0.5

* Source: table 21, section 6, Medical Report.[3]
† Average of 28 normal Japanese men, September 1945.

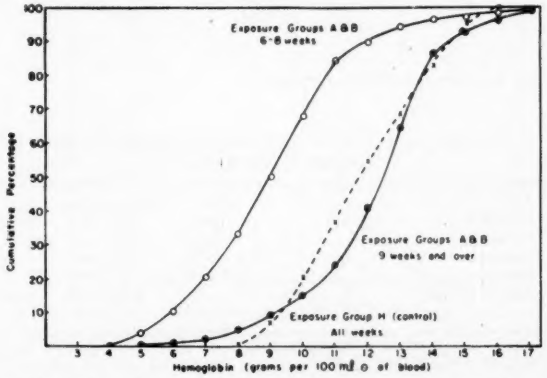

Fig. 2.—Cumulative percentage distribution of hemoglobin values.

Differential Counts.—The most satisfactory group of differential counts available is shown in table 11. These differential counts were made at the same time, and on the same persons, as the counts comprising curve II of figure 1, i. e., persons from Hiroshima in exposure groups A and B who had specific signs of radiation injury. In this table both absolute numbers and percentages of each cell type are shown. The most significant feature of these data is the fact that the absolute number of lymphocytes had not returned to normal by the twelfth week, whereas the granulocytic cells reached normal levels in the eighth to ninth week.

Detailed analyses of the various cell types revealed no other significant or useful trends. The eosinophilia which was observed, particularly among citizens of Nagasaki, was apparently not related to the atomic

bomb, since it appeared in control groups to an even greater extent than in exposure groups A and B.

Erythrocyte and hemoglobin variations in patients with severe radiation injury were less consistent than the changes in the leukocytes. It appeared that the lowest values occurred in weeks 6 to 8 after the bombing and that the return toward normal was evident after the ninth week. The data most useful for purposes of illustration are shown in figure 2. It will be seen that in the control group (H) 50 per cent of the normal subjects had values for hemoglobin concentration of less than

TABLE 12.—*Distribution of Reticulocyte Counts in Patients with Severe Radiation Injury, Weeks 3 to 4 After the Bombing (JAP)**

Fatal Cases	% Reticulocytes	Nonfatal Cases
9	0 to 0.4	6
0	0.5 to 0.9	4
0	1.0 to 2.0	2
0	Over 2.0	1
9		13

* Source: table 5, section 6, Medical Report.[2]

TABLE 13.—*Mean Red Cell Diameters of Patients with Severe Radiation Injury (JC)**

Source	Week	Mean Diameter, Microns †	Number
Hiroshima	3 to 4	7.02 ± 0.312	6
Nagasaki	4 to 5	7.28 ± 0.610	10
Nagasaki	9 to 10	7.85 ± 0.428	20
Normal Japanese	.....	7.52 ± 0.292	23

* All measurements were made by Major Samuel Berg, Medical Corps, Army of the United States. Source: table 9, section 6, Medical Reports.[2]
† Mean ± standard error of the mean.

11.5 Gm. These low values are probably a reflection of the poor nutritional state of the residents of the bombed cities.

Reticulocytes.—A small number of reliable reticulocyte counts was performed, the results of which are shown in table 12.

Red Cell Size.—Measurements of the diameter of erythrocytes were made, using blood films obtained from typical patients with severe radiation injury. The results are shown in table 13.

The Price-Jones curves for the last two groups (table 14) are shown in figure 3. The difference in the mean red cell diameters between patients convalescent from severe radiation injury and normal Japanese controls from the same vicinity is significant.

Corpuscular Constants.—The values for mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration were determined on

samples of blood from a group of patients convalescent from severe radiation injury during weeks nine to 12 after the bombing. The results of this examination are shown in table 14.

These studies of cell size indicate that during the recovery period after radiation injury the red cells are larger than normal. It is assumed that this is due to the occurrence of a reticulocytosis at this time.

Thrombocytes and Clotting Studies.—A considerable number of thrombocyte counts is available, as well as a lesser number of valid studies

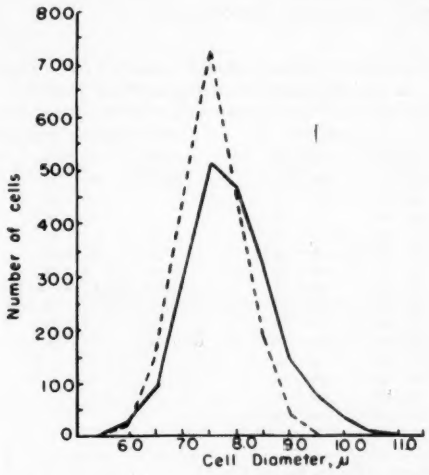

Fig. 3.—Price-Jones curves for patients with severe radiation injury during weeks 9 to 10 after the bombing (solid line) and for normal Japanese controls (broken line).

TABLE 14.—*Red Blood Cells of Patients with Severe Radiation Injury Weeks 9 to 12 After the Bombing (JC)**

No.	Source	Mean Cell Volume,† Cubic Microns	Mean Cell Hemoglobin,† Micromicrograms	Mean Cell Hemoglobin Concentration,† %
66	Nagasaki	93.1 ± 1.31	31.2 ± 0.51	33.2 ± 0.45
55	Controls	89.1 ± 1.35	31.2 ± 0.37	33.9 ± 0.27

* Source: table 8, section 6, Medical Report.[2]
† Mean ± standard error of the mean. The difference between the means of patients and controls is significant in the case of mean cell volume and mean cell hemoglobin.

of the clotting mechanism. The significant data for thrombocytes are shown in table 15.

TABLE 15.—*Distribution of Thrombocyte Counts in Patients with Severe Radiation Injury During Weeks 3 to 6 (JAP)**

Fatal Cases	Thrombocytes	Nonfatal Cases
10	Less than 25,000	3
2	25,100 to 50,000	8
0	50,100 to 100,000	5
0	100,100 to 150,000	5
0	150,100 to 200,000	1
0	More than 200,000	2
12		24

* Source: tables 27 and 28, section 6, Medical Report.[2]

TABLE 16.—*Relationship Between Leukocyte Count and Hemorrhagic Tendency in Patients Examined in Fourth Week (JAP)**

White Blood Cell Count, Less Than 1,000				White Blood Cell Count, 1,000 to 2,000				
Rumpel-Leede Reaction	Bleeding Time, Min.	Bleeding Time, Sec.	Clotting Time, Min.	Clotting Time, Sec.	Rumpel-Leede Reaction	Bleeding Time, Min.	Bleeding Time, Sec.	Clotting Time, Min.	Clotting Time, Sec.
+	28	00	13	30	—	16	30	..	..
+	23	00	12	30	—	11	30	12	30
+	30	00	16	30	—	6	30	12	30
+	13	30	13	30	—	10	00	14	00
+	30	30	15	00	—	4	30	11	30
+	22	30	15	30	White Blood Cell Count, 2,000 to 3,000				
—	20	00	12	30	—	8	30	13	00
—	17	00	15	00	—	7	00	9	00
—	16	00	14	00	—	5	00	13	00
—	24	30	..	..					
—	19	30	20	30					
—	26	30	10	30					
—	20	00	8	30					
—	3	30	..	..					

* Source: table 32a, section 6, Medical Report.[2]

TABLE 17.—*Study of the Hemorrhagic Tendency in the Severe Type of Radiation Injury (JAP), Average Values for Seven Patients**

Week After Bombing	Thrombocytes	Bleeding Time,† Min.	Bleeding Time,† Sec.	Clotting Time,‡ Min.	Clotting Time,‡ Sec.	Capillary Fragility §	
						Infraclavicular Region	Volar Area of Arm
4	105,000	12	45	6	30	16.0	16.5
5	81,300	8	20	6	45	17.3	16.6
6	90,800	4	00	5	30	18.8	19.6
7	82,000	2	45	5	15	22.0	22.2
8	75,000	3	00	4	30	23.5	23.5
Normal	250,000 to 300,000	2 to 3	00	3 to 4	00	More than 25 to 26 mm. Hg	

* Source: table 33, section 6, Medical Report.[2]
† Bleeding time determined by Duke's method.
‡ Clotting time determined by capillary tube method.
§ Capillary fragility determined by vacuum method.

The relationship between the defect in the clotting mechanism and the injury to the bone marrow as reflected by the leukocyte count is well shown in table 16. The fate of these particular patients is not known, but they were selected for study during the fourth week because they

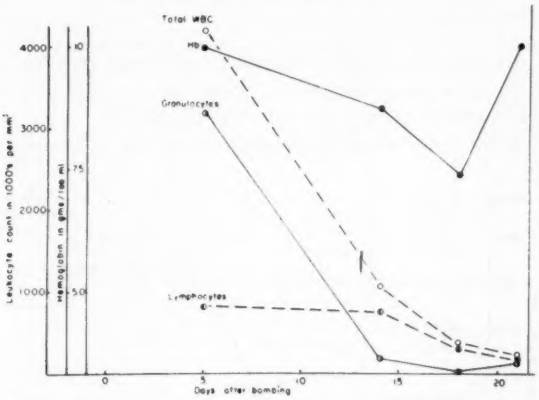

Fig. 4.—Serial hemograms of patient (case 4, Nakao) with typical fatal severe radiation injury.

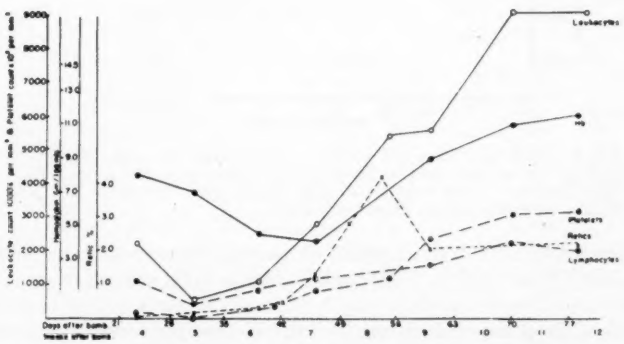

Fig. 5.—Serial hemogram of typical nonfatal case (patient S. K., a man 23 years of age, from the Kusunoki series) of severe radiation injury.

obviously had severe radiation injury. All had spontaneous purpura, and 13 of the 22 had experienced epilation at that time.

The only reliable serial study of the hemorrhagic tendency was made on a group of patients with severe radiation injury who recovered and

who were studied for five weeks. The results of this study are shown in table 17. The hematological changes in patients with severe radiation injury form a characteristic pattern which is consistent with the other clinical findings. To complete this section, attention is directed to the serial blood counts of a typical fatal case, figure 4, and to those of a typical nonfatal case, figure 5.

MODERATELY SEVERE AND MILD RADIATION INJURY

The Japanese physicians did not devote much attention to patients with milder grades of radiation injury. For purposes of analysis and demonstration, it is desirable to use exposure groups C, D and E. With

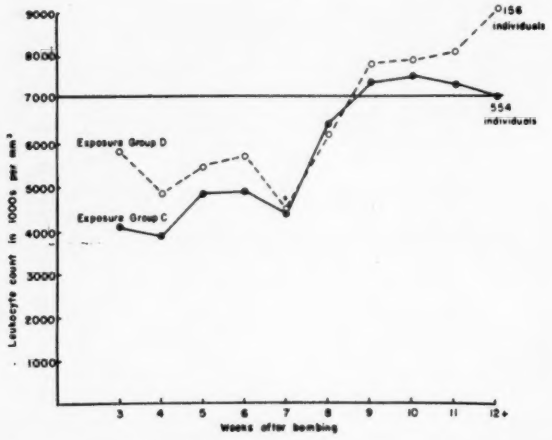

Fig. 6.—Time trend of pooled weekly leukocyte counts in exposure groups with moderately severe and mild radiation injury (source: Table 33N, Section 9, Medical Report [2]). Star indicates average of less than 10 counts. Base line at 7,000 cells represents normal value for Japanese.

reference to table 2, this includes all persons outdoors and in wooden buildings from 1,650 to 3,300 yards (1,500 to 3,000 meters) from zero, persons in heavy buildings from 1,100 to 2,750 yards (1,000 to 2,500 meters) from zero and persons in bomb shelters and tunnels from 0 to 2,200 yards (0 to 2,000 meters) from zero.

The incidence of either epilation or purpura (i. e., specific signs of radiation injury) in these exposure groups was 13.3, 5.8 and 4.1 per cent for Hiroshima, and 21.8, 10.4 and 3.0 per cent for Nagasaki, in groups C, D and E, respectively.

Leukocytes.—In the case of leukocyte counts, there are insufficient data per week to justify considering exposure group E at this time.

Pooled weekly mean leukocyte counts for persons in exposure groups C and D are shown in figure 6. The distribution of the leukocyte counts obtained in weeks two to five for these groups is shown in table 18.

The distribution of leukocyte counts of persons in group H (i. e., persons who received no or insignificant amounts of radiation) is shown

TABLE 18.—*Distribution of Leukocyte Counts During Weeks 2 to 5, Exposure Groups C, D and H: Persons Who Were Living Twenty Days After the Bombing (JC and JAP)**

White Blood Cell Count	Hiroshima			Nagasaki		
	C	D	H	C	D	H
0 to 499	3	0	0	2	2	0
500 to 999	3	1	0	12	1	0
1,000 to 1,999	13	3	0	26	6	0
2,000 to 2,999	12	6	2	30	3	0
3,000 to 3,999	23	11	6	21	5	1
4,000 to 4,999	24	15	20	33	4	3
5,000 to 5,999	29	24	24	30	5	15
6,000 to 6,999	17	18	26	16	7	22
Over 7,000	32	33	34	34	15	53
Mean	5,010	5,870	6,366	4,447	5,408	7,961
Pooled groups C and D		5,370			4,630	

* Source: tables 34H and 34N, section 9, Medical Report.[2]

TABLE 19.—*Pooled Mean Differential Counts for Persons in Exposure Groups C and D Two to Six Weeks After the Bombing**

Cell Type	Hiroshima		Nagasaki	
	Absolute Count, Mean	Per Cent	Absolute Count, Mean	Per Cent
Neutrophils	3,032	60.1	2,520	57.1
Lymphocytes	1,669	33.1	1,647	37.3
Monocytes	175	3.5	114	2.6
Eosinophils	160	3.2	127	2.9
Basophils	6	0.1	8	0.1
Total	5,042	....	4,416	....
Number of cases	135		14	

* Source: tables 40H and 40N, section 9, Medical Report.[2]

for comparison. Here again, there is no simple obvious explanation for the difference between the means in group H. The differences between the means of groups C and D and of the pooled groups C and D are not significant. The differences between these means and the means of group H are significant. The differential counts for persons in groups C and D for the period two to six weeks after the bombing are shown in table 19.

Erythrocyte and hemoglobin variations in patients with the milder grades of radiation injury are best demonstrated in terms of the cumula-

tive percentage distribution of hemoglobin concentration. The data are shown in figure 7. As in the case of severe radiation injury, the values for erythrocytes and hemoglobin concentration were lowest in the six to eight weeks after bombing and then returned toward normal.

Thrombocyte Counts and Clotting Studies.—There was not a sufficient number of thrombocyte counts recorded for patients with milder grades of radiation injury to justify presenting the data. It is reasonable to assume, however, that the thrombocytopenia in persons of exposure groups C, D and E was less severe than in the more exposed patients. A good indication of the severity of the damage to the clotting mechanism is obtained from a tabulation of symptoms of hemorrhage, purpura and other hemorrhagic manifestations. For the sake of comparison, data for

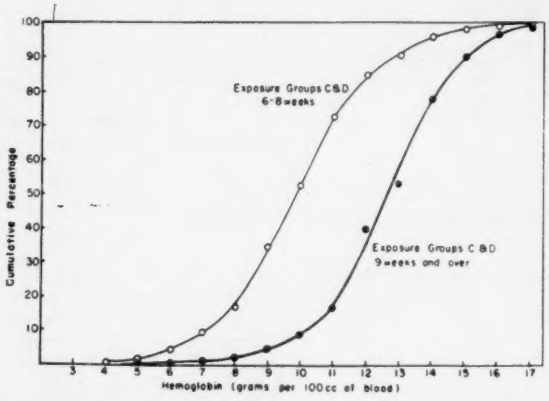

Fig. 7.—Cumulative percentage distribution of hemoglobin values.

the first five exposure groups are shown in table 20. Here again, there is no simple explanation for the discrepancy in the incidence of hemorrhagic manifestations in persons similarly exposed to radiation in the two cities.

Relationship Between Hematological Findings and Clinical Evidence of Radiation Injury.—It was mentioned above that one of the objectives of the Joint Commission's study was to determine the symptomatology of total body radiation injury in man. It is apparent that the changes in the white blood cell count in man are of the same order of magnitude and show the same time trend as have been observed in other large mammals exposed to lethal doses of ionizing radiation. As a result of statistical analysis of symptoms recorded in the questionnaires used, it was possible to define two specific symptoms of radiation injury, epilation

and purpura. It was also possible to define three suggestive symptoms of radiation injury, vomiting on the day of the bombing, oropharyngeal lesions and hemorrhagic manifestations (other than cutaneous purpura). In general, a person with one or both of the specific symptoms had some or all of the suggestive symptoms. It is reasonable to assume that a person with few symptoms either had received less radiation or had a greater resistance to the effects of it than had the person who experienced the complete syndrome. In any case, if the symptoms are a valid indica-

TABLE 20.—*Incidence of Purpura and Other Manifestations of a Hemorrhagic Tendency by Exposure Groups (JC)**

Exposure Group	Hiroshima		Nagasaki	
	Number	Per Cent	Number	Per Cent
A	550	96	248	66
B	485	43	610	42
C	227	12.5	387	24
D	137	8.5	93	13
E	52	7.0	38	6

* Source: tables 23H and 23N, section 9, Medical Report.[2]

TABLE 21.—*Relationship Between Symptoms and Leukocyte Count by Exposure Groups in Weeks 2 to 6 After Bombing**

	Hiroshima				Nagasaki			
	Exposure Groups A and B		Exposure Groups C and D		Exposure Groups A and B		Exposure Groups C and D	
White Blood Cell Count	Under 3,000	Mean	Under 3,000	Mean	Under 3,000	Mean	Under 3,000	Mean
Specific symptoms	%	.....	%	.....	%	.....	%	.....
Epilation and purpura	80.0	1,923	73.3	2,420	78.0	2,181	63.3	2,943
Epilation or purpura	63.4	2,604	29.6	4,167	60.2	3,018	66.2	2,975
Suggestive symptoms only	38.6	3,980	8.6	5,944	31.6	4,511	25.0	5,006
No symptoms	10.6	4,791	6.0	6,368	23.1	5,148	14.5	5,839

* Source: tables 57H and 57N, section 6, Medical Report.[2]

tion of the severity of the radiation injury, there should be a close correlation between them and the leukopenia that occurred. Such a relationship is clearly demonstrated in table 21. These data are important, since they validate mutually not only the significance of the changes in the white blood cell count but also the prescribed symptomatology.

SUMMARY AND CONCLUSIONS

It is possible to describe the syndrome of radiation injury in man in terms of typical clinical symptoms and a typical blood picture. The time trend of the syndrome is important, and the characteristic elements in the diagnosis become apparent at various times after exposure.

In general, the severity of the symptoms paralleled the severity of the leukopenia. Likewise, the important symptoms became evident at about the time the lowest leukocyte count was observed.

The changes in the thrombocytes paralleled those in the leukocytes. The onset and severity of a hemorrhagic tendency followed closely the variations in the thrombocyte count. The alteration in the red blood cells was less consistent and less useful in diagnosis or prognosis than was the time trend of the leukopenia.

From the standpoint of prognosis, the Japanese physicians stated that patients with less than 500 leukocytes per cubic millimeter were least likely to recover. The data presented here neither support nor deny this clinical observation.

In general, the observation quoted at the head of this paper is borne out: "the greater the dose (of radiation), the more profound is the blood damage, the more rapidly it develops and the more slowly it is repaired."

It is apparent that knowledge of the serial variation in the leukocyte count of a victim of radiation injury is of great clinical importance. It is doubtful whether the other elements of the hemogram are of comparable significance.

In the event of an emergency, it would be perfectly justifiable to perform only leukocyte counts and determinations of hemoglobin, hematocrit and protein by the copper sulfate ($CuSO_4$) method. This would be an economical procedure and would provide the type of information required to evaluate individual cases and to aid in control of therapy.

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The Medical Illustration Department, Naval Medical School, Navy Medical Center, prepared the figures.

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HEMOLYTIC ANEMIA SECONDARY TO A FAMILIAL (HEREDITARY) DEFECT IN HEMOGLOBIN SYNTHESIS

Report of a Case with Radioiron Studies

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IN RECENT years there has been increased interest in familial and acquired anemias associated with erythrocytic inclusion bodies.[1] Simultaneously, the hereditary transmission of certain hematological disorders has received attention.[2] The purpose of this report is to present

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1. (*a*) Doniach, I.; Grüneberg, H., and Pearson, J. E. G.: The Occurrence of Siderocytes in Adult Human Blood, J. Path. & Bact. **55**:23 (Jan.) 1943. (*b*) Pappenheimer, A. M.; Thompson, W. P.; Parker, D. D., and Smith, K. E.: Anemia Associated with Unidentified Erythrocytic Inclusion Bodies After Splenectomy, Quart. J. Med. **14**:75 (April) 1945. (*c*) McFadzean, A. J. S., and Davis, L. J.: Iron-Staining Erythrocytic Inclusions with Especial Reference to Acquired Haemolytic Anaemia, Glasgow M. J. **28**:237 (Sept.) 1947; (*d*) On the Nature and Significance of Stippling in Lead Poisoning, with Reference to the Effect of Splenectomy, Quart. J. Med. **18**:57 (Jan.) 1949. (*e*) Mills, H., and Lucia, S. P.: Familial Hypochromic Anemia Associated with Post-Splenectomy Erythrocytic Inclusion Bodies, Blood **4**:891 (Aug.) 1949. (*f*) Crosby, W. H.: Hereditary Non-spherocytic Hemolytic Anemia, ibid. **5**:233 (March) 1950.

2. (*a*) Mills and Lucia.[1e] (*b*)Crosby.[1f] (*c*) Cooley, T. B.: A Severe Type of Hereditary Anemia with Elliptocytosis, Am. J. M. Sc. **209**:561 (May) 1945.

(Footnote continued on next page)

an investigation which has led us to propose that there exists a type of hemolytic anemia secondary to a hereditary defect in hemoglobin synthesis and associated with rubricytic inclusion bodies. It is our opinion that mechanisms heretofore not generally recognized have been demonstrated in this study.

BACKGROUND

A. *Familial Anemias.*—The familial nature of certain anemias is well known. Familial hemolytic anemia (familial spherocytosis) is transmitted as a mendelian dominant trait, and the genetics of hereditary leptocytosis (thalassemia; Mediterranean disease) and sickle cell disease have been clarified recently. Valentine and Neel [2e] postulated that thalassemia major may be due to homozygosity for a factor which when heterozygous results in thalassemia minor, and Neel [2f] advanced the opinion that a similar relationship prevails between sickle cell anemia and sicklemia (sickle cell trait). Pauling and his associates [2g] agreed with Neel and have demonstrated that the erythrocytes of persons with sickle cell anemia contain exclusively a form of hemoglobin which gives an electrophoretic pattern different from that of normal hemoglobin. The cells of subjects with sicklemia contain but 40 per cent of this abnormal hemoglobin designated as "sickle cell anemia hemoglobin." They postulated that the inherited defect in sickle cell disease is an abnormality in the globin portion of the hemoglobin molecule and ascribed thereto the clinical and laboratory findings noted in this condition.

Cooley [2c] reported on a family in which severe hypochromic anemia associated with striking elliptocytosis appeared to be transmitted to the men by unaffected women. Rundles and Falls [2d] studied two families in which the men through several generations were afflicted with hypochromic, microcytic anemia, whereas the women had minor abnormalities, such as ovalocytosis and splenomegaly. The authors considered the anemia to be a sex-linked abnormality, the women carrying the recessive or incompletely recessive gene. Subsequently, Mills and Lucia [1e] reported a similar anemia occurring in maternal half-brothers. More recently, Crosby [1f] studied a family in which nonspherocytic hemolytic anemia appeared to be transmitted as a mendelian dominant trait. All anemic members of this family were of blood group A, and anemia was associated but not genetically linked with brachyphalangia.

B. *Erythrocytes with Iron-Containing Inclusion Bodies—Siderocytes.*—Occurrence in Various Diseases: A classification of erythrocytic

(*d*) Rundles, R. W., and Falls, H. F.: Hereditary (? Sex-Linked) Anemia, ibid. **211**:641 (June) 1946. (*e*) Valentine, W. N., and Neel, J. V.: Hematologic and Genetic Study of the Transmission of Thalassemia, Arch. Int. Med. **74**:185 (Sept.) 1944. (*f*) Neel, J. V.: The Inheritance of Sickle Cell Anemia, Science **110**:64 (July 15) 1949. (*g*) Pauling, L.; Itano, H. A.; Singer, S. J., and Wells, I. C.: Sickle Cell Anemia: A Molecular Disease, ibid. **110**:543 (Nov. 25) 1949.

inclusion bodies has been published recently, and emphasis is placed on those which contain iron, thereby giving a positive prussian blue reaction.[1e] Erythrocytes with inclusion bodies containing iron are called "siderocytes," a name given them in 1941 by Grüneberg,[3] when he described the transient anemia associated with the flexed tail and belly spot phenomenon of mice. Subsequently, many reports have appeared in which significant numbers of siderocytes have been demonstrated, and invariably their concentration increased greatly following splenectomy. Diseases in this group include acquired hemolytic anemia,[4] lead poisoning,[5] Banti's syndrome (postsplenectomy),[1a] familial hemolytic anemia [4b] and the hereditary anemias discussed above. In this laboratory we have found the stippled cell of hereditary leptocytosis to be fundamentally similar.

McFadzean and Davis [1c] studied eight patients with acquired hemolytic anemia in whom postsplenectomy siderocytosis was prominent. These investigators observed relatively small numbers (11 per cent or less) of siderocytes in the peripheral blood of their patients prior to splenectomy, whereas the bone marrow contained large numbers of rubricytes with siderotic granules. After splenectomy, large numbers (16 to 88 per cent) of siderocytes appeared in the peripheral blood of their subjects, and they were of the opinion that the rise in total red blood cell count for a variable period following splenectomy could be accounted for by the increased number of siderocytes appearing in the peripheral blood. They demonstrated siderotic granules within the endothelial cells of the spleen and postulated that the siderocytes were defective cells rapidly removed from the circulation by the reticuloendothelial system, especially the spleen.

More recently, McFadzean and Davis [1d] studied the anemia associated with lead poisoning and demonstrated that the stippled cell of lead poisoning is fundamentally similar to the siderocyte. They observed that human beings afflicted with this disease have many more rubricytes with siderotic granules in the bone marrow than there are stippled cells in the peripheral blood. When lead poisoning was produced in guinea pigs, splenectomy in these animals was followed by a remarkable rise in the number of siderocytes in the peripheral blood. Furthermore, the number of stippled cells in the peripheral blood of guinea pigs whose spleens had been removed and which had been given toxic doses of lead was greatly increased, as compared with control animals not subjected

3. Grüneberg, H.: Siderocytes: A New Kind of Erythrocytes, Nature, London **148**:114 (July 26) 1941.

4. (*a*) McFadzean and Davis.[1e] (*b*) Dacie, J. V., and Doniach, I.: The Basophilic Property of the Iron-Containing Granules in Siderocytes, J. Path. & Bact. **59**:684 (Oct.) 1947.

5. (*a*) McFadzean and Davis.[1d] (*b*) Case, R. A. M.: Siderocytes in Hæmolytic Diseases, J. Path. & Bact. **57**:271 (April) 1945.

to splenectomy but given similar doses of lead. Invariably, stippled rubricytes could be demonstrated in the bone marrow before the peripheral blood contained stippled cells. They concluded that lead poisoning is a hemolytic anemia, and that lead toxicity in some way alters hemoglobin formation by the rubricytic nucleus, resulting in the production of defective erythrocytes that are easily destroyed.

Splenectomy performed for sex-linked familial anemia was followed by the appearance of many erythrocytes containing "Howell-Jolly bodies" in one patient studied by Cooley [2e] and by prominent siderocytosis in the two men reported on by Mills and Lucia [1e] and in two men from Rundles and Falls' series.[2d] Mills and Lucia postulated that the anemia in their patients was secondary to an inherited defect in iron metabolism manifested by the production of abnormal erythrocytes, namely, siderocytes, but did not suspect a hemolytic anemia because in neither case was there noted reticulocytosis, increased fragility, jaundice or increased urobilinogen excretion in the urine; the icterus index and serum bilirubin were normal in the one instance in which these tests were done.

One patient described by Crosby [1f] had a rise in peripheral blood siderocyte count from 0.1 to 45 per cent following splenectomy. Cross cell survival studies revealed that this patient's erythrocytes had a decidedly short survival as compared with those of the normal control. Evidence was presented that a heat-labile hemolytic factor was present in this man's serum, and increased fecal urobilinogen excretion and reticulocytosis were constant findings.

Morphologic Aspects: The morphologic appearance of the siderocytes noted by various authors is essentially similar.[6] These cells contain from one to more than 10 siderotic granules, the average number per cell usually increasing for a variable period following splenectomy. The granules vary from 0.5 to 2.0 microns in diameter and are usually coccoid, although many shapes have been noted. Diffuse stippling is sometimes seen, and occasionally masses of inclusion bodies will reduce the hemoglobin content of the siderocyte to a thin peripheral rim. In the bone marrow granules are seen only within rubricytes showing evidence of hemoglobin formation. They are located adjacent to the nucleus in the immature rubricytes, but as hemoglobin formation and cell maturation progress they tend to be more peripheral, the location most commonly seen in the peripheral blood siderocyte. They usually stain blue or bluish purple in Wright-Giemsa preparations and when treated for 20 minutes with 2 per cent potassium ferrocyanide and 2 per cent hydrochloric acid solutions give a positive prussian blue reaction, signifying the presence of iron. Siderotic granules are anisotropic and gram-negative, give a negative Feulgen reaction (for nucleic acid), do not stain with hema-

6. Footnote 1 *b* to *f*. Grüneberg.[3] Dacie and Doniach.[4b] Case.[5b]

toxylin, do not fix complement and contain iron that is not in the form of ferritin or hemosiderin.[1b] All granules within siderocytes do not give a positive prussian blue reaction. It has been our experience that granules adjacent to the rubricytic nucleus are more likely to react positively than those which are more peripheral; the possible significance of these observations will be discussed later.

REPORT OF CASE

Present Illness.—D. F. H., a 48 year old divorced white man of English-Dutch extraction, was first seen at the San Francisco Veterans Administration Hospital in May 1947. His chief complaint was easy fatigability associated with nervousness and left-sided tinnitus. The nervousness and fatigability began eight months previously, after he learned that he was anemic. The tinnitus began in 1943, having developed when he was near a large gun that was fired. He had lost approximately 15 pounds (6.8 Kg.) but at no time had he noticed any other symptoms that might be referable to an anemia, although he had been regarded as pale all his life. He had lived in another city, and the physician who previously cared for him wrote recently that he was given an iron preparation for anemia in 1937 and 1940; the records of his hemogram during this period were not available. However, this physician repaired an inguinal hernia in 1942, at which time the hemoglobin concentration was recorded as "75 per cent." In the same year he was commissioned in the United States Army. Physical examination recorded no significant disabilities, but no blood count was done. In November 1945, when he entered an Army hospital for treatment of an inguinal hernia, the result of a hemoglobin examination (Sahli) was recorded as 11.6 Gm. per 100 ml. In January 1946, when he entered another hospital for repair of the hernia, the erythrocyte count was reported as 3,200,000 per cubic millimeter and the hemoglobin concentration as 10.5 Gm. per 100 ml. In February 1946 an erythrocyte count of 3,360,000 per cubic millimeter and a hemoglobin concentration of 11.5 Gm. per 100 ml. were recorded. In April 1946 he was discharged from the Army with a diagnosis of "idiopathic macrocytic anemia."

Family History.—The patient's father died at the age of 72, after six years of illness with swollen ankles, weakness, dyspnea and an anemia diagnosed as "pernicious anemia." Liver extract therapy had no effect on his anemia. The patient's mother died at the age of 49 of cancer, the primary site of which was unknown. He had no male siblings but had two sisters who were in fairly good health. One sister, aged 52, had the following hemogram: a hemoglobin concentration of 82 per cent and 4,260,000 red blood cells and 7,600 white blood cells per cubic millimeter, with 56 per cent segmented neutrophils, 1 per cent band neutrophils, 40 per cent lymphocytes, 2 per cent eosinophils and 1 per cent monocytes. The second sister, aged 58, had the following hemogram: a hemoglobin concentration of 11.6 Gm. per 100 ml. and 3,840,000 red blood cells and 5,650 white blood cells per cubic millimeter, with 31 per cent segmented neutrophils, 2 per cent band neutrophils, 62 per cent lymphocytes and 5 per cent monocytes. Both sisters lived out of the state and, unfortunately, were not available for study by us. The patient had a 15 year old daughter who was under treatment for anemia in another city, but her hemogram could not be obtained.

Past History and System Review.—He denied any use of toxic drugs and had had no episodes of blood loss. He had had no significant illnesses except chronic neurodermatitis of both legs of six years' duration, acute appendicitis in 1937 and

a recurrent inguinal hernia for which herniorrhaphies were performed in 1920, 1942 and 1946.

Results of Physical Examination.—The patient was a well developed, slightly pallid white man, with no significant lymphadenopathy. There was a neurodermatitis on the anterior aspect of the lower part of the legs. The eyes, ears, nose and throat were normal except for bilateral nerve type deafness with moderate diminution of hearing. The heart, lungs and abdomen were normal. The liver, spleen and kidneys were not palpably enlarged. Rectal examination showed no abnormality except for three small hemorrhoidal tags. The extremities were normal; there was no clubbing or brachyphalangia. Neurological examination gave normal results except for bilateral eighth nerve deafness.

Laboratory Data.—The red blood cell count varied from 2,500,000 to 3,350,000 per cubic millimeter on repeated analyses between 1947 and 1950. The hemoglobin concentration similarly varied between 9.5 and 11 Gm. per 100 ml. The mean cell volume was 111 cubic microns; mean cell hemoglobin measured 36 micromicrograms, with a mean cell hemoglobin concentration of 32 per cent. White blood cells numbered 6,900 per cubic millimeter, with 51 per cent segmented neutrophils, 1 per cent band neutrophils, 42 per cent lymphocytes, 4 per cent monocytes and 2 per cent eosinophils; there were 242,000 platelets (thrombocytes) per cubic millimeter. Repeated reticulocyte counts revealed values varying from 0.2 to 2.5 per cent, but the great majority were less than 1.5 per cent. Examination of the blood smear (fig. 1 *G*) revealed moderate anisocytosis and poikilocytosis. There were occasional ovalocytes, macrocytes and target cells. Rare stippled cells were seen (less than 0.5 per cent). Osmotic fragility tests failed to show any decreased or increased resistance as compared to normal controls. Using a modification of Crosby's technic to test for mechanical fragility,[2b] a 10 per cent solution of the patient's cells was placed with beads in a serology tube and shaken for three hours in a Kahn shaker; normal controls were similarly treated. After centrifugation of the tubes, the hemoglobin in the supernatant fluid was measured, and the values found for the patient did not differ significantly from those for the controls. A 10 per cent solution of the patient's cells was incubated for 18 hours with the following material: (1) patient's plasma, (2) patient's plasma inactivated at 56 C. (133 F.) for 10 minutes, (3) inactivated plasma with complement added and (4) homologous plasma similarly treated. The mixtures were centrifuged, and the amount of hemoglobin in the supernatant fluid was determined. This varied from 15 to 45 mg. per 100 ml. and was not significantly different from values obtained in normal controls run concomitantly. The patient's serum complement titer compared to that of controls was normal. The Coombs test for blocking antibodies gave a negative reaction, and no sickling or acid hemolysis was demonstrated. Bleeding time, clotting time and clot retraction were normal. His blood was type A, Rh positive. Repeated urinalyses showed no abnormality, and Fischer's test [7] for coproporphyrin revealed no abnormal excretion. The 24-hour excretion of urobilinogen in the urine varied between 1.5 and 3.5 mg. The fecal urobilinogen excretion varied between 72 and 168 mg. per 24 hours. Repeated stool examinations for occult blood and for pathogenic ova and parasites were negative. The icterus index was 5.4 units; serum bilirubin in one minute measured 0.06 mg. and in 30 minutes 0.42 per 100 ml. The total serum protein was 6.5 Gm. per 100 ml., with 3.8 Gm. of albumin and 2.7 Gm. of globulin. The test with sulfobromophthalein (bromsulphalein®) sodium U. S. P.

7. Fischer, H., cited by Vannotti, A.: Porphyrine und Porphyrinkrankheiten, Berlin, Julius Springer, 1937, p. 270.

(5 mg. per kilogram of body weight) showed no retention in 45 minutes. Cephalin flocculation was normal. No serum agglutinins were demonstrated against typhoid, paratyphoid and Brucella organisms. The Kahn and Kolmer tests gave negative reactions. Gastric analysis revealed 86 degrees of free hydrochloric acid following administration of histamine. The roentgenologic appearance of the chest, of the bones, including the hands, and of the upper and lower parts of the gastrointestinal tract was repeatedly normal. The splenic shadow was not enlarged.

Course.—At present the patient is working as a salesman and "feels fine" except for nervousness under stress. He is able to climb the San Francisco hills without undue dyspnea, and in general he is as active as he was 10 years ago. The following therapy has failed to alter his anemia: administration of (1) iron orally and intravenously, (2) liver extract intramuscularly and intravenously, (3) folic acid, (4) vitamin B_{12} and (5) massive doses of all the common vitamins.

SPECIAL STUDIES

I. *Bone Marrow.*—A. The marrow was cellular and easily obtained.

B. The differential count was as follows[8]:

Cells	%
Progranulocytes	0.5
Myelocytes (neutrophilic)	9.0
Myelocytes (eosinophilic)	0.5
Metamyelocytes (neutrophilic)	14.5
Metamyelocytes (eosinophilic)	1.0
Band neutrophils	18.5
Segmented neutrophils	14.0
Prorubricytes	1.0
Rubricytes (basophilic)	2.5
Rubricytes (polychromatic)	13.5
Rubricytes (normochromatic)	13.0
Metarubricytes	4.0
Plasmacytes	1.5
Lymphocytes	6.5

C. Counts were made to determine the percentage of stippled rubricytes present in the Wright-Giemsa preparations and to determine the percentage of rubricytes containing siderotic granules when the prussian blue technic was employed. In each instance 30 to 40 per cent of the rubricytes contained readily distinguishable granules (fig. 1).

II. *Blood Volume.*—By the P^{32} tagged cell method described by Berlin and his associates,[9] the circulating red cell mass was 1.52 liters and plasma volume 2.98 liters. By extrapolation of the radioiron plasma clearance curve, the volumes were 1.58 and 3.07 liters, respectively.

8. The terminology used throughout this paper is that recommended by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs (Blood **4**:89 [Jan.] 1949). A major change is the use of the term "rubricyte" instead of "normoblast."

9. Berlin, N.; Lawrence, J. H., and Gartland, J.: The Blood Volume Determinations in Polycythemia as Determined by P^{32} Labelled Red Blood Cells, Am. J. Med., to be published.

III. *Plasma Iron.*—On two occasions the plasma iron was 2.0 and 2.2 micrograms per milliliter by the method of Kitzes and his co-workers.[10] (The average normal value is 1.4 micrograms per milliliter.)

IV. *Rate of Plasma Iron Turnover.*—The rate of plasma iron turnover is defined as the quantity of iron entering or leaving the plasma per unit time. To determine this rate, a tracer dose of radioiron (Fe^{59}; containing an amount of iron insignificant when compared with total plasma iron) is incubated for 20 minutes with

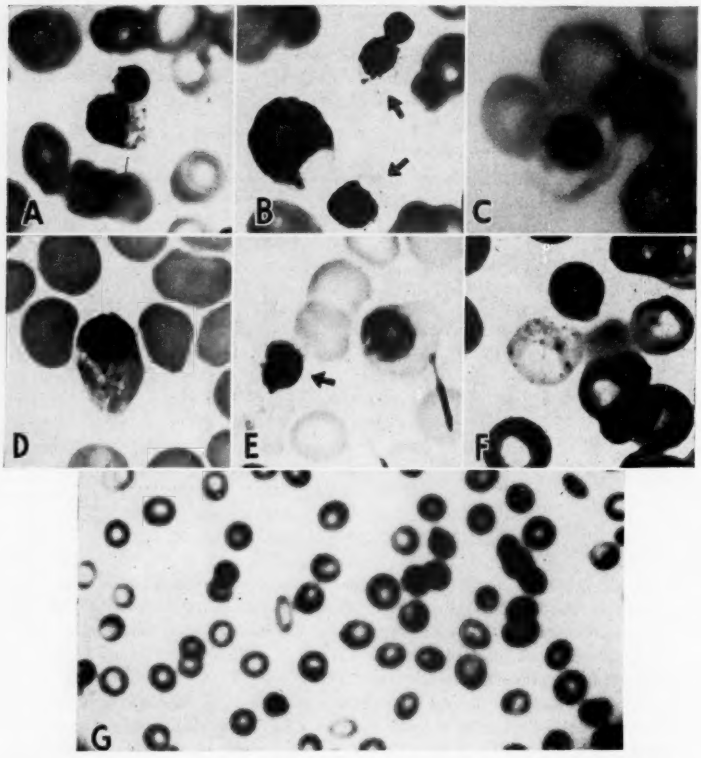

Fig. 1.—*A*, *B* and *C* are photomicrographs of bone marrow smears stained with Wright-Giemsa stain (× 1,164). Note tendency for peripheral stippling and vacuolation in rubricytes. *D* and *E* are bone marrow smears treated for prussian blue reaction and counterstained with safranine (× 1,164). Note granules giving positive prussian blue reaction with tendency to paranuclear location. *F*, siderocyte in peripheral blood smear (× 1,164). *G*, peripheral blood smear demonstrating moderate anisocytosis, poikilocytosis, occasional ovalocytosis and target cells (× 570).

10. Kitzes, G.; Elvehjem, C. A., and Schuette, H. A.: Determination of Blood Plasma Iron, J. Biol. Chem. **155**:653 (Oct.) 1944.

approximately 20 ml. of the patient's plasma to allow combination with the iron-binding globulin. This mixture is then injected intravenously, and the blood samples are taken at approximately hourly intervals for radioiron analysis. Since the plasma iron level remains relatively stable during the experimental period, the rate constant of the radioiron can be applied with simplicity in calculating the total plasma iron exchange rate. The half-time of plasma iron turnover in this patient was 1.2 hours, which is about 20 per cent less than normal (fig. 2). With a plasma iron content of 2 micrograms per milliliter and a plasma volume of 2.98 liters, the above time constant gives a plasma iron turnover rate of 83 mg. per day, or 1.1 mg. per kilogram of body weight per day. The corresponding value on 17

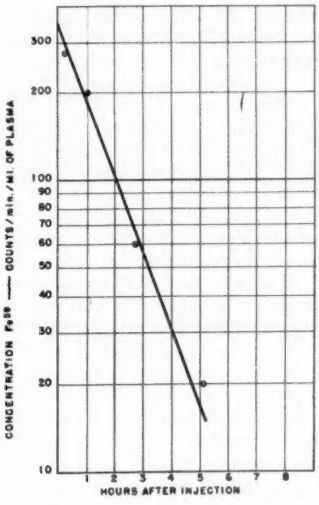

Fig. 2.—Time concentration function of a single intravenous tracer dose of radioiron in a patient having hemolytic anemia secondary to defect in hemoglobin synthesis.

normal male subjects was found to be 0.55 mg. per kilogram of body weight per day. The method of calculation has been described [11] and is as follows:

$$\frac{0.693}{1.2\text{ hr.}} \times 2.0\text{ micrograms/ml.} \times 2{,}980\text{ ml.} \times 24\text{ hr.} = 83\text{ mg./day}$$

Where $\frac{0.693}{1.2\text{ hr.}}$ represents the absolute value of slope of the line shown in figure 2 and is the characteristic rate constant. The number 0.693 (natural log of 2) is chosen arbitrarily depending on the time unit used. In this case it is the half-period.

11. Huff, R. L.; Hennessy, T. G.; Austin, R. E.; Garcia, J. F.; Roberts, B. M., and Lawrence, J. H.: Plasma and Red Cell Iron Turnover in Normal Subjects and in Patients Having Various Hematopoietic Disorders, J. Clin. Investigation, to be published.

V. *Rate of Red Cell Iron Turnover.*—The rate of red cell iron turnover is defined as the quantity of iron entering red blood cells per unit time. To determine this, blood samples are taken at frequent intervals for the first 14 days after the injection and the cells analyzed for radioiron. The amount of the injected tracer iron present in our patient's cells at maximum concentration was 40 per cent, compared with the average for normal men of 75 per cent. Since plasma iron is removed at a relatively constant rate by various tissues, such as the bone marrow and iron storehouses, it is thought that a close approximation of red cell iron turnover rate can be computed from the product of the plasma iron turnover rate and the fraction of the tracer iron present in red cells at maximum concentration, or

$$83 \text{ mg./day} \times 0.40 = 33.2 \text{ mg./day.}$$

This is near the average for 17 normal men, 24.0 mg. per day. However, in the presence of a constantly low red cell mass, the fraction of iron turned over per unit time is high (total red cell iron determinations revealed 1 mg. of iron per cubic centimeter of packed cells).

$$\frac{33.2 \text{ mg./day} \times 100}{1{,}520 \text{ mg. Fe in red cell mass}} = 2.2\% \text{ per day.}$$

This value in 17 normal subjects was 1.05 per cent, ± 0.21 standard deviation. In the absence of iron exchange of cells other than during formation or destruction, the mean cell life of this subject was then 45 days, as compared with the normal for this technic of 95 days.

Figure 3 illustrates a second point which is thought to be significant. There is a flattening of the uptake curve lying outside the range of normal between the fourth and eighth days. In time relationship this corresponds to what is normally the most active period of hemoglobin formation in the marrow. Thus, the initial slope of the line does not differ significantly from the normal, for it represents the radioiron in erythrocytes produced by precursors (reticulocytes and metarubricytes) whose function of hemoglobin synthesis had been nearly completed when the radioiron was injected. However, during the fourth to eighth days the slope of the uptake curve represents the erythrocytes produced by the cells (basophilic, polychromatic and normochromatic rubricytes) which were most actively engaged in hemoglobin synthesis at the time the radioiron was injected. The depression of the slope at this point is thought to be the result of an increased rate of destruction of the abnormal cells (siderocytes) produced in our patient. The low percentage utilization of radioiron (40 per cent) as compared with the average normal (75 per cent) is considered to be a reflection in part of the patient's high total plasma and storage iron, resulting from previous administration of large doses of iron intravenously and orally. Thus, if a constant amount of iron is utilized for production of erythrocytes, a smaller percentage of a given tracer dose will appear in erythrocytes in the presence of increased body iron. However, it is probable that rapid destruction of siderocytes accounts for a significant part of the apparent low total utilization.

COMMENT

Hereditary Nature of the Anemia.—It is our opinion that this patient is afflicted with a chronic refractory anemia of familial (hereditary) origin. The evidence for considering this a familial anemia is as follows: (1) the possibility that there existed a similar type of anemia in his father, (2) the history of anemia in his daughter, (3) the mild anemia

noted in one of his sisters and (4) the striking similarity between this patient's hematologic findings and those noted in persons with familial anemias discussed elsewhere in this article. That this disease may have existed from childhood is indicated by (1) the absence of the patient's exposure to toxins and our failure to demonstrate any antibodies similar to those seen in acquired hemolytic anemia, (2) the known chronicity without essential change for 14 years and (3) the lack of significant symptoms secondary to his anemia, suggesting that he is accustomed to this state. On occasion he has had mild fatigability, but this began after the anemia was first discovered and has disappeared since the patient was reassured regarding his anemia.

It is our opinion that the type of anemia present in our patient falls into the group of familial anemias described by Crosby,[1f] Mills and Lucia,[1e] Cooley[2c] and Rundles and Falls,[2d] although certain genetic

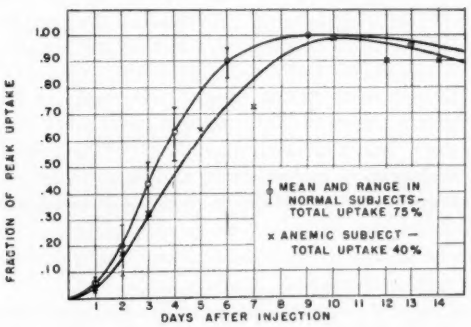

Fig. 3.—Time concentration curves of radioiron in red cells of normal male subjects and patient having hemolytic anemia secondary to defect in hemoglobin synthesis.

differences are recognized. In the family studied by Crosby, the anemia was transmitted as a mendelian dominant trait, whereas sex linkage to the female was noted by the other investigators. Yet certain common characteristics are thought to be present. These are (1) the northern European extraction, (2) a chronic anemia of the nonspherocytic type, (3) the absence of definite signs linking these anemias to hereditary leptocytosis and (4) the presence of erythrocytic and/or rubricytic inclusion bodies containing iron. Crosby suggested that the anemia in his family group most closely resembled congenital hemolytic icterus (spherocytosis). In our patient basophilic stippling and the occasional target cell seen in the peripheral blood resemble observations in hereditary leptocytosis, although it should be emphasized that rubricytic stippling is more prominent and target cells less prominent than are

usually seen in hereditary leptocytosis. The northern European extraction, the normal osmotic fragility reactions, the high mean cell volume and lack of hypochromia are findings which exclude this condition from the group of hereditary leptocytosis. At present it seems prudent not to form any specific classification for these unusual anemias. Rather, it seems more important to recognize that familial anemias occur which do not fall into any group of commonly accepted hereditary anemias.

Hemolytic Mechanism Resulting from Defective Hemoglobin Formation Due to Altered Exchange of Iron Between the Nucleus and the Cytoplasm of the Rubricyte.—If hemolytic disease is defined as one characterized by an increased rate of red blood cell destruction, it is our opinion that the subject of this report is suffering from a hemolytic anemia. The increased rate of red cell iron turnover and the short mean cell life, indicated by the radioiron studies, seem to be adequate proof of this. Furthermore, the large number (30 to 40 per cent) of stippled rubricytes in the marrow in contrast to the very small number (less than 0.5 per cent) of siderocytes seen in the peripheral blood is indicative of a hemolytic mechanism that destroys most of the erythrocytes derived from these unusual stippled rubricytes. In a similar case Crosby [1f] found a short cell life by the technic of cross transfusion cell survival studies, and McFadzean and Davis [1c, d] have demonstrated a hemolytic mechanism in lead poisoning and acquired hemolytic anemia, instances in which an acquired defect results in a hemolytic mechanism similar in certain respects to that noted in our patient. It seems likely that similar hereditary anemias, such as described by Mills and Lucia,[1e] are also hemolytic in nature.

It is postulated that the primary defect in such patients is an inherent one concerning the passage of iron between cytoplasm and nucleus, resulting in faulty hemoglobin synthesis. In support of this, there are presented experimental data for physiologic iron turnover in the nuclei of the marrow of normal rabbits.

Turnover studies on the nuclei and cytoplasm of the bone marrow of rabbits indicate that there normally may be a period during which tracer iron in the marrow enters into nuclei. In figure 4 are presented the data from the following experiment. Rabbits were given tracer quantities of Fe^{55} and Fe^{59} intravenously and were killed at intervals during the first 19 hours after injection. Marrow was collected from both hind extremities, usually about 5 Gm. per animal. An aliquot was taken for analysis of radioactivity per unit weight of marrow. The remainder of the sample was subjected to separation of nuclei and cytoplasm by the method of Mirsky and Pollister.[12] The radioactivity contained in separated cytoplasmic and nuclear fractions from an aliquot of marrow was determined. Figure 4 *A* shows the radioactivity per unit weight of total marrow. The highest

12. Mirsky, A. E., and Pollister, A. W.: Chromosin, Desoxyribose Nucleoprotein Complex of the Cell Nucleus, J. Gen. Physiol. **30**:117 (Nov.) 1946.

concentration is reached in about seven hours. In contrast, the highest concentration of radioiron in nuclei (fig. 4 *B*) occurred at about two to four hours and represented 8 per cent of the tracer in marrow. There is insufficient data (total iron determinations of nuclei and cytoplasm of blood-free marrow) to calculate the amount of iron passing through the nuclei, but it is possible that all the iron may have a nuclear transition phase. Because of the variation of the initial determinations on individual rabbits, confirmation must await experiments now in progress.

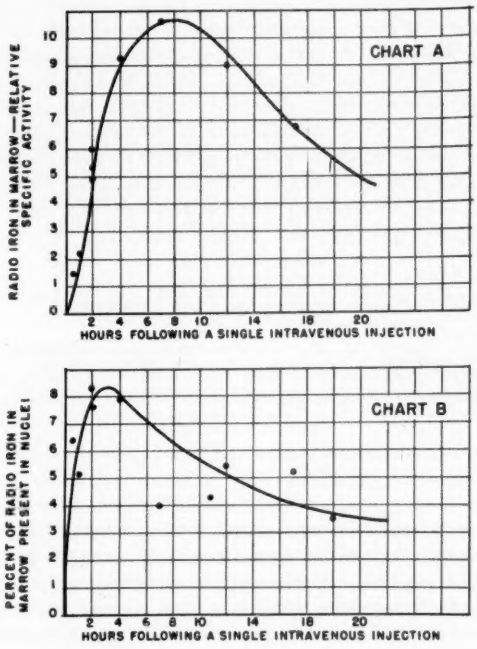

Fig. 4.—Time concentration curves of radioiron in the bone marrow of rabbits (chart A) and in bone marrow nuclei (chart B) following a single intravenous injection.

In the case under discussion, we propose that the manifestations of the defect in the mechanism of iron exchange between cytoplasm and nucleus are (1) initial accumulation of siderotic granules at the paranuclear zone, (2) cells poor in hemoglobin, (3) pronounced vacuolation, (4) rapid iron turnover rates, and (5) defective cells with a short peripheral life.

Even though the location of the metabolic defect is granted, the mechanism involved remains obscure. It appears that nuclear iron turn-

over is necessary in the synthesis of hemoglobin, but the underlying abnormality causing the defect is not known. It may reside within the nucleus, in the cytoplasm or in both, or it may be in some precursor, or in a foreign interfering substance.

It is believed that an important expression of this defect in hemoglobin synthesis is the "piling up" of iron within the rubricyte. This "piling up" process becomes obvious when granules giving a positive prussian blue reaction are demonstrated (fig.1). Siderotic granules are first seen in rubricytes showing evidence of hemoglobin formation, tending to be adjacent to the nucleus in the immature cells and becoming more peripheral with maturation. When the prussian blue technic is employed, most of the granules giving a positive reaction are centrally located, whereas in the Wright-Giemsa preparation the stippling is more generalized and tends to be peripheral; this phenomenon is considered to occur as the result of a combination between iron and some organic compound occurring as the rubricyte matures. Thus, in prussian blue preparations the granules most likely to give a positive

Location of Granules in Stippled Rubricytes

	Central (Paranuclear)	Generalized	Peripheral
Wright-Giemsa stain	14%	44%	42%
Fractions that are vacuolated	1/10	⅛	¼
Prussian blue reaction	50%	30%	20%

reaction are adjacent to the nucleus and are generally in the immature cells. In contrast, the Wright-Giemsa stain is fixed to granules giving a positive prussian blue reaction, as well as to those which do not; thus, the more numerous mature cells tend to have peripheral stippling because of granules that in many instances no longer give a positive prussian blue reaction. It seems less likely that a free globin-porphyrin complex and/or an aggregation of stromal material could account for some of the basophilic stippling noted in the Wright-Giemsa preparations. Further evidence of the defect in hemoglobin synthesis is the striking vacuolation noted in many of the rubricytes. This vacuolation increases with maturation and may be so pronounced that the hemoglobin content of the cell is reduced to but a thin rim. It is especially prominent in stippled rubricytes and in general parallels peripheral stippling. These phenomena are best illustrated in the above table.

The usual criteria for the diagnosis of hemolytic disease are anemia, reticulocytosis, an elevated serum bilirubin content and increased excretion of urobilinogen in the feces. The findings of increased osmotic fragility, spherocytosis and circulating antibodies are corroborative. If one applied the usual criteria to our patient, the diagnosis of a hemo-

lytic anemia would be missed, for repeated studies have failed to reveal reticulocytosis, an elevated serum bilirubin value, increased urobilinogen excretion, increased red cell fragility or circulating antibodies. Our explanation for this is based on the assumption that the inherent defect results in the production of defective cells poor in hemoglobin, although rich enough in iron to give an increased rate of red cell iron turnover. These cells, the siderocytes, are destroyed soon after reaching the circulation. The persistent lack of reticulocytosis (this patient has had approximately 40 normal reticulocyte counts during the past five years) would indicate either a rapid destruction of the reticulated cells or the inability of the marrow to respond to the anemia with a reticulocytosis. There is neither elevation of the serum bilirubin level nor increased urobilinogen excretion because the destroyed cells contain little hemoglobin, and, therefore, it would require destruction of large numbers of these cells to release abnormally large quantities of pigment. Only slightly accelerated destruction of siderocytes would thus be sufficient to cause anemia without an unusual increase in pigment production. The hemolytic index was calculated [13] $\left(\frac{\text{daily fecal urobilinogen output} \times 100}{\text{total circulating hemoglobin}}\right)$ using data from simultaneous determinations of the blood volume, hemoglobin concentration and fecal urobilinogen excretion. The values obtained varied from 14 to 35 compared with the normal of 11 to 21. This indicates that periodically the amount of pigment released from destroyed erythrocytes is sufficiently increased to give a hemolytic index slightly greater than usual, a further indication of a hemolytic process.

In our patient it appears that approximately one third of the rubricytes are producing defective cells, the siderocytes, while the erythrocytes derived from the remaining rubricytes are larger (mean cell volume, 111 cubic microns) and contain more hemoglobin (mean cell hemoglobin, 36 micromicrograms) than normal erythrocytes. Preliminary studies in our laboratory indicate that similar mechanisms may exist in some cases of hereditary leptocytosis, and it appears likely that other congenital and acquired hemolytic anemias may fall into a related category.

Management.—It is our opinion that the management of the anemia seen in our patient must be conservative. In one child with severe hereditary anemia associated with elliptocytosis, splenectomy appeared to be of definite value.[2c] However, in at least three instances in which splenectomy was performed on persons suffering from anemia similar to that seen in our patient, gradual deterioration and death ensued.[2a, d] In four patients splenectomy resulted in no fundamental change of

13. Miller, E. B.; Singer, K., and Dameshek, W.: Use of the Daily Fecal Output of Urobilinogen and the Hemolytic Index in the Measurement of Hemolysis, Arch. Int. Med. **70**:722 (Nov.) 1942.

status.[14] Because splenectomy would result only in the decreased rate of destruction of the poorly hemoglobinated siderocytes without fundamentally affecting the hereditary defect, it seems plausible that this procedure would be of little value, and, furthermore, the tendency to postoperative thrombocytosis would arise with the threat of thrombosis, such as occurred in the two subjects studied by Mills and Lucia.[1e] Treatment with the usual antianemia preparations has been of little value in our patient as in other similar patients. Secondary hemosiderosis may follow the administration of iron for a prolonged period. Similarly, multiple transfusions can result in hemosiderosis and perhaps a hemochromatosis-like state.[15] Our patient's state of well-being might temporarily be improved by transfusions, but we are loathe to submit him to the hazard of hemosiderosis since he is not incapacitated by his disease. It is our opinion, therefore, that at present reassurance and explanation offer the most potent therapeutic agents in this condition.

SUMMARY

A case is presented in which it is proposed that there exists a hemolytic anemia secondary to a hereditary defect in hemoglobin synthesis and associated with rubricytic inclusion bodies. There has been demonstrated a hemolytic mechanism in which the usual laboratory findings seen in hemolytic diseases are absent because of defects in hemoglobin formation. It is postulated that the primary defect is an inherent one concerning the exchange of iron between cytoplasm and nucleus; this results in the production of defective cells poor in hemoglobin.

Iron turnover data on marrow and marrow nuclear material of rabbits are presented. These data indicate that there is normally a phase during which iron passes through nuclei.

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14. Crosby.[1f] Rundles and Falls.[2d] Estren, S., and Dameshek, W.: Current Concepts of Hemolytic Anemias, in Dock, W., and Snapper, I.: Advances in Internal Medicine, New York, Interscience Publishers, Inc., 1949, vol. 3, p. 90.

15. Schwartz, S. O., and Blumenthal, S. A.: Exogenous Hemochromatosis Resulting from Blood Transfusions, Blood **3**:617 (June) 1948. Wyatt, J. P., and Goldenberg, H.: Hemosiderosis in Refractory Anemia, Arch. Int. Med. **83**:67 (Jan.) 1949. Muirhead, E. E.; Crass, G.; Jones, F., and Hill, J. M.: Iron Overload (Hemosiderosis) Aggravated by Blood Transfusions, ibid. **83**:477 (May) 1949.

INSULIN RESISTANCE

Review of the Literature and Report of a Case Associated with Carcinoma of the Pancreas

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INSULIN resistance has been arbitrarily defined as a state which requires 200 or more units per day for longer than 48 hours for regulation in a nonacidotic person with diabetes mellitus.[1] Diabetic acidosis and acute infectious processes are well known causes of temporary increase in the daily insulin requirement, but such cases are not classified as true resistance to the hormone. Absolute refractoriness to insulin probably does not exist as an entity, since administration of large quantities of the substance has always resulted in a definite and frequently adequate therapeutic response. Sheppard[2] reported on a patient in diabetic coma who was given 56,080 units of insulin over a 26 hour period, with subsequent regulation on 40 units of protamine zinc insulin and 60 units of regular insulin each day. His case, though striking, constituted only a temporary increase in insulin requirement due to diabetic acidosis and was not a true case of insulin resistance according to the criteria previously noted. Smelo's report[3] of a young female diabetic who required 1,250,000 units of insulin during a four year period, an average dosage of 850 units per day, represents the most remarkable case of sustained tolerance to the hormone which has thus far appeared in the literature.

From the Medical Services, Emory University Hospital and Grady Memorial Hospital.

1. (*a*) Root, H. F.: Insulin Resistance and Bronze Diabetes, New England J. Med. **201**:201, 1929. (*b*) Martin, W. P.; Martin, H. E.; Lyster, R. W., and Strouse, S.: Insulin Resistance: Critical Survey of the Literature with Report of a Case, J. Clin. Endocrinol. **1**:387, 1941.

2. Sheppard, J. G. H.: Diabetic Coma Treated with 56,000 Units of Insulin, Brit. M. J. **1**:576, 1949.

3. Smelo, L. S.: Lack of Response to Insulin: Report of a Patient Treated with 5,000 Units per 24 Hours, South. M. J. **40**:333, 1947.

Insulin resistance has been reported in association with several disease entities, but there have been no constantly associated pathological changes. The present communication is an attempt to tabulate recorded cases briefly and to describe a case of insulin-resistant diabetes with a previously unreported associated disease, carcinoma of the pancreas.

REPORT OF CASE

First Admission.—M. S. was a 49 year old Negro woman who was originally admitted to Grady Memorial Hospital in diabetic acidosis on Sept. 14, 1948. She had noted polyuria, polydipsia, polyphagia and increasing weakness with weight loss of about 25 pounds (11.3 Kg.) during the preceding three months. Nine months prior to admission she had noted onset of vague gnawing epigastric pain which occurred 10 to 15 minutes after ingestion of any type food and was not relieved by alkalis. There was no family history of diabetes.

Remarkable physical findings were moderate obesity (weight, 175 pounds [79.4 Kg.]), lethargy, moderate dehydration, blood pressure of 150/110, mild sclerotic changes in the arterioles of the ocular fundus, slight enlargement of the heart to the left and large external and internal hemorrhoids.

Laboratory Data: The urine had a specific gravity of 1.030 and contained sugar (4+), acetone (2+) and albumin (1+). There was no anemia. Blood sugar on her admission was 309 mg. and cholesterol 252 mg. per 100 cc. The Kahn reaction was negative. The guaiac test for blood in the feces gave a positive result.

She was rendered acetone free in seven hours with administration of 450 units of regular insulin and 7,000 cc. of 5 per cent dextrose in isotonic sodium chloride solution intravenously. Because the presence of a gastrointestinal neoplasm was suspected, a series of gastrointestinal roentgenograms, visualization with barium sulfate enema and contrast visualization of the gallbladder were done, and all organs were within normal limits. The roentgenographic appearance of the chest was normal. The patient had cystitis due to Escherichia coli while in the hospital; this cleared promptly after administration of 1 Gm. of sulfadiazine orally three times each day. Because of the obesity she was given an 800 calory diet; 20 units of protamine zinc insulin was to be taken each morning. She was discharged on her seventeenth hospital day.

She was followed in the diabetic clinic until Dec. 1, 1948, by which time she had lost an additional 40 pounds (18.1 Kg.) and her insulin dosage had been reduced to 15 units of the protamine zinc preparation daily. On her routine visit to the clinic on December 1 she was found to have a 4+ reaction for urinary sugar and a 3+ reaction for urinary acetone. Admission to the hospital was arranged.

Second Admission.—Her only complaint at this time was vague epigastric distress which had been present for four days. The blood pressure was 160/100, and she appeared to have lost weight (weight, 134 pounds [60.8 Kg.]); otherwise there were no significant changes in physical findings.

Laboratory data revealed urinary sugar (4+) and urinary acetone (3+), 5,000,000 red cells per cubic millimeter of blood, a hemoglobin concentration of 12 Gm. per 100 cc. of blood, an erythrocyte sedimentation rate of 55 mm. per hour and a white cell count of 8,900 per cubic millimeter of blood, with a normal differential count. Her blood sugar was 415 mg., cholesterol 241 mg. and non-

protein nitrogen 24 mg., per 100 cc. Plasma protein was 6.0 Gm. per 100 cc.; the reaction to the formol-gel test was negative, and the cephalin cholesterol flocculation was 2+. Thymol turbidity was 1 unit, the icterus index 11 units and the prothrombin time normal; the guaiac test for blood in the feces gave a consistently positive reaction.

Four hours after her admission to the hospital the patient's urine was acetone free following administration of 275 units of regular insulin and 5,500 cc. of fluid. She was given a 1,000 calory diabetic diet. On the second and third hospital days she received 40 and 45 units of insulin, respectively, yet glycosuria (4+) persisted. On the fourth hospital day she again had acetonuria (1+), which was controlled with 75 units of regular insulin and orange juice. On the fifth hospital day she again had a 1+ reaction for urinary acetone, which required 100 units of insulin for control. At this time the patient was afebrile, and extensive search for infection revealed nothing. With the possibility of hyperfunction of the adrenal cortex in mind as a possible cause of increased insulin requirement, a total circulating eosinophil count (Dunger method) was obtained. This count was 100 per cubic millimeter, which is within normal limits.

Evidence that the patient was resistant to insulin appeared on the sixth hospital day. Ketosis again was seen; the blood sugar was 416 mg. per 100 cc.; the carbon dioxide-combining power was 38 volumes per cent, and the urine gave a 2+ reaction for acetone. Acetonuria disappeared after the patient had received 675 units of regular insulin over a 17 hour period. During the subsequent three days she was given 215, 680 and 340 units of insulin, respectively. Despite this dosage she had acetonuria on several occasions but remained acetone free most of the time. During the evening of the ninth hospital day, at which time she had 4+ reaction for urinary sugar and a faint trace of acetone, she surreptitiously left the hospital, and all attempts to locate her were without avail.

About 28 hours later she was brought back in deep coma. The blood pressure was 120/80, and the pulse rate was 140 per minute. There was mild cyanosis of the mucous membranes. The white blood cell count was 21,000 per cubic millimeter, and the urine contained sugar (4+) and acetone (4+).

The patient was given a total dose of 9,600 units of regular insulin over the following 17 hour period, six doses of which were of 1,000 units each intravenously. Isotonic sodium chloride and lactated Ringer's solutions were given alternately for the first 10 hours without change in the urinary sugar. These were followed by infusions of 5 per cent dextrose solution, until a total of 17,000 cc. of fluid, containing a total of 550 Gm. of dextrose, had been given. Sixteen grams of potassium chloride was administered intravenously during that period. The patient became acetone free 17 hours after her admission. Urinary output was profuse; glycosuria persisted until death, and no evidence of pulmonary edema appeared. Cyanosis persisted despite intranasal oxygen administration; the blood pressure remained at 120/80; the pulse rate stayed at about 140 per minute, and the coma deepened progressively until death occurred 27 hours after admission.

During the episode of resistance two commercial brands of insulin were used with no detectable difference. There was never any evidence of local or systemic reaction to the injection of insulin.

Clinical Diagnoses.—Final clinical diagnoses were (1) insulin-resistant diabetes mellitus, (2) diabetic coma and (3) probable neoplasm of gastrointestinal tract.

Test for Insulin-Neutralizing Antibodies in Patient's Serum.—Serum obtained from the patient on the day before her death and four days previously was pooled

and tested for an insulin antagonist in mice according to the method of Lowell.[4] Six groups of five mice each were used. Five-tenths cubic centimeter of the patient's serum, with 0.03 unit of regular insulin added, produced convulsions in all mice more rapidly than did a similar amount of control serum, with the same amount of insulin added. Saline solution and insulin also produced convulsions. After injection of 0.5 cc. of the patient's serum alone into five mice, four convulsed, demonstrating the fact that fairly large amounts of insulin were circulating at the time the blood specimens were drawn. Injection of control serum and saline solution alone did not produce convulsions. It is probable that large amounts of circulating insulin will prevent detection of neutralizing antibodies, so it cannot be concluded that failure to demonstrate them in this case absolutely rules out their presence.

Autopsy Observations.—Postmortem examination was done 6½ hours after death. The pancreas weighed 120 Gm., was club shaped and pinkish tan and contained within the body a firm mass measuring 7.5 by 7.5 by 5.0 cm. On cut section the parenchyma of the head was lobulated and normal in appearance. The firm mass in the body on cut section appeared to have larger lobules, was grayish white and contained numerous hemorrhagic areas throughout. On microscopic examination a section of the mass revealed a highly undifferentiated adenocarcinoma which was invading lymphatics and blood vessels. Section of the tail of the pancreas revealed an acute and chronic pancreatitis of long standing with conspicuous fibrosis and no remaining normal acinar tissue. A few islets of Langerhans were still present and were normal in appearance.

The liver weighed 1,730 Gm. and on cut surface was smooth, glistening and reddish brown. Scattered over the surface of the liver were several firm white nodules measuring 0.5 to 2.5 cm. in diameter. On microscopic section these nodules were seen to be metastatic adenocarcinoma with pronounced stromal proliferation.

The heart weighed 400 Gm. There were moderate arteriosclerotic changes in the coronary arteries and aorta. Microscopic section revealed a small myocardial infarction which appeared to be two to three days old. There was degeneration of the muscle fibers, with loss of nuclei and striation. Interstitial edema and acute inflammatory infiltration in the areas of necrotic muscle were present. Several small areas of old myocardial fibrosis were seen throughout the section.

The left adrenal gland weighed 6 Gm. and the right 5 Gm. The microscopic section showed congestion and serious depletion of lipid in the zona fasciculata.

The pituitary was normal in size and shape. On microscopic section there were normal numbers of chromophobe, eosinophilic and basophilic cells in the anterior portion, and no abnormalities were seen.

The brain showed pronounced depletion of the small round cells in the cerebellar cortex, and many of those present appeared to be degenerating.

Final Anatomic Diagnoses.—The final anatomic diagnoses were insulin-resistant diabetes mellitus with diabetic coma (clinical), atypical encephalomalacia of the cerebellum, carcinoma of the body of the pancreas with metastases to the liver, acute and chronic pancreatitis, cardiac hypertrophy, small myocardial infarction about three days old and generalized arteriosclerosis.

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4. Lowell, F. C.: Immunologic Studies in Insulin Resistance: II. The Presence of a Neutralizing Factor in the Blood Exhibiting Some Characteristics of an Antibody, J. Clin. Investigation **23**:233, 1944.

COMMENT

Carcinoma of the pancreas associated with insulin-resistant diabetes has not been previously reported. A patient (case 826) of Joslin [5] manifested increased requirements for insulin; her diabetes was "mild for many years and then suddenly required during the last few months of her life from 50 to 100 units of insulin a day and at autopsy a large carcinoma of the pancreas was found." Urmy, Jones and Wood [6] observed a case of diabetes mellitus and fatty diarrhea which required 90 units of insulin each day for one month for regulation and then required 40 units every day until death, with autopsy revealing extensive colloid adenocarcinoma of the pancreas, chronic interstitial pancreatitis, abscess of the pancreas and generalized peritonitis. Grafe [7] cited a case of extensive carcinoma of the pancreas which resulted in mild diabetes.

Depancreatized man probably requires only 35 to 50 units of insulin daily to control the resultant diabetes,[8] so that some factor other than destruction of islet tissue by adenocarcinoma or the concomitant acute pancreatitis is required to explain this patient's resistance to insulin. No circulating antibodies to insulin were demonstrated. It is not believed that carcinoma per se accounted for the resistance, and it is preferable to classify this case as one of insulin resistance due to unknown factors.

REVIEW OF LITERATURE

In a thorough search of the literature 50 cases which met the criteria for insulin-resistant diabetes mellitus were collected and are tabulated in table 1. None of these patients was under undue stress, such as infectious processes or surgical procedures, and each required at least 200 units of insulin per day for two consecutive days while in a non-acidotic state. Insulin resistance is uncommonly encountered and must

5. Joslin, E. P.: The Treatment of Diabetes Mellitus, ed. 4, Philadelphia, Lea & Febiger, 1928.

6. Urmy, T. V.; Jones, C. M., and Wood, J. C.: A Case of Diabetes Mellitus and Fatty Diarrhea Due to Carcinoma of the Pancreas, Am. J. M. Sc. **182**:662, 1931.

7. Grafe, E.: Metabolic Diseases and Their Treatment, Philadelphia, Lea & Febiger, 1933, p. 249.

8. Waugh, J. M.; Dixon, C. F.; Clagett, O. T.; Bollman, J. L.; Sprague, R. G., and Comfort, M. W.: Total Pancreatectomy: A Symposium Presenting 4 Successful Cases and a Report on Metabolic Observations, Proc. Staff Meet., Mayo Clin. **21**:25, 1946. Ricketts, H. T.; Brunschwig, A., and Knowlton, K.: Effects of Total Pancreatectomy in a Patient with Diabetes, Am. J. Med. **1**:229, 1946. Goldner, M. G., and Clark, D. E.: The Insulin Requirement of Man After Total Pancreatectomy, J. Clin. Endocrinol. **4**:194, 1944. Brunschwig, A.; Ricketts, H. T., and Bigelow, R. R.: Total Pancreatectomy, Total Duodenectomy, Splenectomy, Left Adrenalectomy and Omentectomy in a Diabetic Patient with Recovery, Surg., Gynec. & Obst. **80**:252, 1945.

TABLE 1.—*Summary of Data on Insulin-Resistant Patients as Reported in the Literature*

Authors	Case No.	Age, Yr.	Greatest 24-Hour Dose, Units	Duration	Manifestations of Allergy	Insulin-Neutralizing Antibodies in Serum	Associated Diseases	Autopsy Observations
Glassberg, B. Y.; Somogyi, M., and Taussig, A. E.: Arch. Int. Med. **40**: 676 (Nov.) 1927	1	"Young"	1,100	1 yr.	Local induration	None detected	None apparent	.....
Taussig, A. E.: Tr. A. Am. Physicians **42**: 166, 1927	2	"Young"	1,100	1 mo.	Localized and generalized	.....	None apparent	.....
Sztavrovszky, P., and Waltner, K.: Monatsschr. f. Kinderh. **37**: 214, 1927	3	12	300	Several wk.	None	.....	None apparent	.....
Lawrence, R. D.: Quart. J. Med. **21**: 359, 1928	4	17	400	3 mo.	Eosinophilia	.....	None apparent	.....
Byworth, H. A.: Brit. M. J. **1**: 801, 1928	5	23	200	48 days	.....	.....	Pulmonary tuberculosis	.....
Ulrich, H.: Arch. Int. Med. **41**: 875 (June) 1928	6	24	360	1 mo.	.....	.....	Acromegaly	.....
Root [1a]	7	52	1,680	1 mo. (died)	.....	.....	Hemochromatosis	Hemochromatosis
Allen, J. N., and Constam, G. R.: M. Clin. North America **12**: 1677, 1929	8	58	1,000	3 mo. (died)	.....	None detected	Hemochromatosis	Hemochromatosis
Mason, E. H.: J. Clin. Investigation **9**: 31, 1930	9	40	2,075	8 mo. (died)	.....	.....	.....	Small fibrotic pancreas with calcareous degeneration; cyst in midbrain
Mohler, H. K., and Goldburgh, H. L.: M. Clin. North America **15**: 343, 1931	10	72	1,150	40 days (died)	.....	.....	Tuberculosis; hepatomegaly; ? cirrhosis	Autopsy not permitted
Karr, W. G.; Scull, C. W., and Petty, O. H.: J. Lab. & Clin. Med. **18**: 1203, 1933	11	58	600	6 mo.	Local; positive Prausnitz-Küstner reaction	None detected	None apparent	Note: No insulin excreted in urine
Thannhauser, S. J., and Fuld, H.: Klin. Wehnschr. **12**: 252, 1933	12	65	1,230	2 mo.	.....	.....	Slight hepatomegaly	.....
Engel, R.: Klin. Wehnschr. **13**: 1682, 1934	13	..	1,365	....	.....	.....	Hemochromatosis	.....
Hills, R. F.; Sharpe, J. C., and Gay, L. N.: Bull. Johns Hopkins Hosp. **55**: 193, 1934	14	58	890	1½ mo.	.....	.....	Postsubtotal thyroidectomy for thyrotoxicosis	.....
Clay, R. D., and Lawrence, R. D.: Brit. M. J. **1**: 697, 1935	15	63	960	5 days (died)	.....	.....	None apparent	Slight fatty infiltration of liver; old anterior myocardial infarct
Wayburn, E.: Am. J. M. Sc. **190**: 157, 1935	16	44	415	9 days (died)	.....	.....	Pulmonary tuberculosis	Pulmonary tuberculosis
Marble, A.: Arch. Int. Med. **62**: 432 (Sept.) 1938	17	35	675	4 yr.	Eosinophilia	None detected	Rheumatoid arthritis	Note: No insulin found in urine
Wiener, H. J.: Am. J. M. Sc. **196**: 211, 1938	18	58	3,250	9 mo.	Negative Prausnitz-Küstner reaction	None detected	None apparent	.....
Glen, A., and Eaton, J. C.: Quart. J. Med. **7**: 271, 1938	19	48	1,050	3 yr. (died)	.....	Present	None apparent	Fatty degeneration of liver

Regan, J. F.; Westra, J. J., and Wilder, R. M.: New England J. Med. **223**: 745, 1940	20	53	1,720	25 days (died)	Slight local	.....	Chronic cholecystitis with cholelithiasis; terminal bronchopneumonia	Chronic cholecystitis with cholelithiasis; terminal bronchopneumonia
Hart, J. F., and Vicens, C. A.: J. Clin. Endocrinol. **1**: 399, 1941	21	60	92	7 wk.	Local and general	.....	None apparent	.....
Martin and others [4b]	22	53	1,205	11 days (died)	Generalized urticaria	None detected	Congestive heart failure; bronchopneumonia; peritonitis	Necrosis of islets of Langerhans; acute peritonitis; hypernephroma
Strouse, S.; Martin, W.; Martin, H. E., and Lyster, R. W.: Tr. A. Am. Physicians **56**: 61, 1941	23	16	2,100	1 mo.	.....	Present	None apparent	.....
Levi, J. I., and Friedman, H. T.: New England J. Med. **225**: 975, 1941	24	64	1,621	47 days	.....	None detected	Chronic lymphatic leukemia	.....
Wayburn, E., and Beckh, W.: J. Clin. Endocrinol. **2**: 511, 1942	25	64	2,460	4 mo. (died)	.....	Present	Pulmonary tuberculosis	Pulmonary tuberculosis
Lozinski, E., and Frohlich, L. I.: Canad. M. A. J. **46**: 62, 1942	26	61	5,780	9 mo.	.....	None detected	Bilateral hydronephrosis	.....
Glass, W. I.; Spingarn, C. L., and Pollack, H.: Arch. Int. Med. **70**: 221 (Aug.) 1942	27	60	2,795	6 mo.	Negative Prausnitz-Küstner and precipitin reactions	None detected	None apparent	.....
Goldner and Ricketts [28]	28	57	1,000	6 mo. (died)	Urticaria; positive Prausnitz-Küstner and precipitin reactions	None detected	.....	Arteriosclerotic heart disease
Schloss, J. J.: Ann. Int. Med. **19**: 533, 1943	29	18	1,650	10 mo.	.....	None detected	Occasional furuncle	.....
Schreier, H.: New York State J. Med. **43**: 1341, 1943	30	58	1,310	54 days (died)	Urticaria	Present	.....	Fatty degeneration of liver; hyaline degeneration of islets of Langerhans
Pullen, R. L., and Sodeman, W. A.: J. Clin. Endocrinol. **3**: 345, 1943	31	58	260	45 days	.....	.....	Suspected Cushing's disease	.....
Jordan [15]	32	65	627	18 days (died)	.....	.....	"Inflammatory" arthritis with destruction of head of right femur	.....
Shepardson, H. C.; Goble, G., and Withrow, P. B.: California & West. Med. **60**: 201, 1944	33	67	1,580	2 days (died)	Urticaria	.....	Myocardial infarction after onset of resistance	Autopsy not permitted
Lerman, J.: Am. J. M. Sc. **207**: 354, 1944	34	..	700	6 yr.	Local; positive Prausnitz-Küstner and precipitin reactions	"Equivocal results"	Rheumatoid arthritis	.....
Lawrence, R. D.: Lancet **1**: 724 and 773, 1946	35	26	2,160	3 yr. (died)	.....	None detected	Lipodystrophy	Portal cirrhosis with fatty infiltration of liver and spleen; no subcutaneous fat

TABLE 1.—*Summary of Data on Insulin-Resistant Patients as Reported in the Literature—Continued*

Authors	Case No.	Age, Yr.	Greatest 24-Hour Dose, Units	Duration	Manifestations of Allergy	Insulin-Neutralizing Antibodies in Serum	Associated Diseases	Autopsy Observations
McGavack, T. H.; Klotz, S. D.; Vobel, M., and Hart, J. F.: J. Clin. Endocrinol. **6**: 747, 1946	36	65	910	7 wk. (died)	Localized and generalized positive Praus-nitz-Küs-ner reaction	None detected	Portal cirrhosis; acute and chronic pancreatitis	.....
Felder, L.: J. Clin. Endocrinol. **6**: 339, 1946	37	79	1,200	30 days (died)	.....	Present	Dry gangrene of foot	Hyaline degeneration of islets of Langerhans
Smelo [3]	38	17	5,000	4 yr. +	Localized: positive precipitin reaction	Present	None apparent	.....
Lowell, F. C.: J. Clin. Investigation **26**: 57, 1947	39	45	2,900	3 yr. (died)	Urticaria	Present	Pulmonary tuberculosis; chronic pyelonephritis	Autopsy not permitted
Axelrod, A. R.; Lobe, S.: Orten, J. M., and Myers, G. B.: Ann. Int. Med. **27**: 555, 1947	40	60	2,150	6 mo.	.....	None detected	None apparent	.....
.....	41	42	740	15 days	.....	None detected	None apparent	.....
.....	42	54	200	....	.....	None detected	Paresis	.....
Berry, G. M., and Helwig, F. C.: Am. J. Med. **4**: 923, 1948	43	65	5,820	24 days (died)	.....	.....	.....	Acute disseminated pancreatitis; cirrhosis of liver
Scriver, W. de M.: Canad. M. A. J. **58**: 506, 1948	44	41	1,000	6 days (died)	.....	.....	Following thyroidectomy for hyperthyroidism	Hyalinization of some islets of Langerhans; only small amount of thyroid tissue left
Haunz, E. A.: Arch. Int. Med. **83**: 515 (May) 1949	45	63	620	5 mo. (died)	Generalized urticaria	None detected	Arteriosclerotic heart disease	Fatty degeneration of liver; arteriosclerotic heart disease
Joslin, E. P.; Root, H. F.; White, P.; Marble, A., and Bailey, C. C.: The Treatment of Diabetes Mellitus, ed. 8, Philadelphia, Lea & Febiger, 1946, p. 379	46	30	910	1 yr. (?)	.....	.....	None apparent	.....
.....	47	33	2,312	11 mo. (died)	.....	.....	.....	Fatty cirrhosis of liver; pancreatitis; calculi with fibrosis of pancreas
.....	48	40	2,100	2 yr.	Localized	.....	Rheumatoid arthritis	.....
.....	49	39	600	2 yr.	.....	.....	None apparent	.....
.....	50	62	2,900	4 yr.	.....	Present	Lipodystrophy	.....

be distinguished from conditions causing fundamentally high insulin requirements, such as occur in juveniles with severe diabetes, who not infrequently need as much as 100 units of insulin per day for regulation; it must also be distinguished from the conditions causing temporarily increased insulin requirements which are recorded in table 2.

Ten cases previously listed in the literature as of true insulin resistance were not included in this review because they did not meet the criteria previously noted. Eight cases excluded were previously tabulated in the review of Martin and his associates.[1b] Murphy's two patients [9] had allergic reactions to insulin but were never given over 10 units per day. Rudy's patient [10] received more than 200 units of insulin for only one day. Three cases reported by Labbé [11] were not included because the first was of a patient with diabetic acidosis who received over

TABLE 2.—*Conditions Causing Temporary Increase in Insulin Requirement*

I.	Poor absorption from subcutaneous tissues
	Insulin lipodystrophy (lipohypertrophy and lipoatrophy with fibrosis)
	Shock
	Congestive heart failure
II.	Hypermetabolism
	Hyperthyroidism
III.	Insulin deficiency plus hyperfunction of adrenal cortex (alarm reaction of Selye)
	Diabetic acidosis
IV.	Hyperfunction of adrenal cortex
	Surgical procedures
	Trauma
	Acute infectious processes
	Pituitary basophilism
	Carcinoma of adrenal cortex
V.	Increased mobilization of hepatic glycogen
	Pheochromocytoma

200 units of insulin on only one day and died the next; the second was of a patient in diabetic acidosis associated with suppurative gangrene of the foot, lymphangitis and amputation, and the third was of a patient with diabetic acidosis associated with an abscess of the right thigh. Claar's case [12] was one of diabetic acidosis. The case reported by Cannavò [13] was

9. Murphy, J. A.; Beardwood, J. T., and Miller, M. M.: Report on Insulin Sensitivity with Attempt at Passive Transfer, J. Allergy **5**:606, 1934.

10. Rudy, A.: Urticaria and Insulin Resistance with Reference to the Relation of the Skin to the Carbohydrate Metabolism: Report of a Case and Review of the Literature, New England J. Med. **204**:791, 1931.

11. Labbé, M.; Boulin, R., and Balmus: Lésions dégénératives du foie et coma diabétique insulino-résistant, Presse méd. **43**:1105, 1935. Labbé, M., and Boulin, R.: Coma diabétique insulino-résistant, J. de méd. de Paris **54**:471, 1934; Comas diabétiques insulino-résistants, Bull. et mém. Soc. méd. d. hôp. de Paris **47**:943, 1931.

12. Claar, E., and Malcangi, L.: L'insulinoresistenza: Contributo clinico, Riforma med. **53**:1471, 1937.

13. Cannavò, L.: Insulinoresistenza e irradiazioni röntgen della regione ipofisaria, Policlinico (sez. prat.) **43**:1099, 1936.

one of diabetic acidosis with malignant growth of the right thigh and secondary infection. Pollack and Long [14] classified their case as one of insulin resistance, although the patient died in diabetic acidosis on the second day of treatment and on autopsy was observed to have complete thrombosis of the celiac and hepatic arteries. Jordan's second case [15] was not included because the patient had an abscess of the left foot and underwent amputation at the same time increased amounts of insulin were being given.

INCREASED REQUIREMENTS FOR INSULIN

As a result of poor absorption of the substance from the injection site, increased amounts of the hormone are required on injection into fatty or fibrous tissue occurring in insulin lipodystrophy [16] and in circulatory inefficiency which occurs in shock or congestive heart failure. In lipodystrophy the site chosen for injection should be changed to one of normal subcutaneous tissue, and in shock or congestive failure the intravenous route of administration is preferable until a more compensated hemodynamic state can be established.

Owing to increased metabolic demands, hyperthyroidism causes an increased requirement for insulin which may be corrected by control of the thyrotoxicosis.

Pheochromocytoma, presumably because of mobilization of large amounts of glucose from hepatic glycogen, causes an intensification of the diabetic state. Frequently surgical removal results in a dramatic decrease in the daily insulin requirement.[17]

A relationship between function of the pituitary and adrenal glands and the action of insulin has been known since Houssay's classic experiments.[18] Young [19] caused diabetes by injection of anterior pituitary

14. Pollack, H., and Long, E. R.: Thrombosis of the Hepatic Artery with Sudden Resistance to Insulin in a Diabetic Patient, Arch. Path. **13**:530 (March) 1932.

15. Jordan, W. R.: Insulin Resistance: Report of 2 Cases, South. Med. & Surg. **106**:361, 1944.

16. Goldner, M. G.: Insulin Lipohypertrophy, J. Clin. Endocrinol. **3**:469, 1943.

17. deVries, A.; Rachmilewitz, M., and Schumert, M.: Pheochromocytoma with Diabetes and Hypertension: Report of 2 Cases Cured by Operation, Am. J. Med. **6**:51, 1949.

18. Houssay, B. A.: A Group of Lectures on Pituitary Physiology Comprising the Dunham Lectureship Series Delivered at the Harvard Medical School in November, 1935, New England J. Med. **214**:913, 961, 1023, 1086 and 1128, 1936.

19. Young, F. G.: The Identity and Mechanism of Action of the Glycotropic (Anti-Insulin) Substance of the Anterior Pituitary Gland, Biochem. J. **32**:1521. 1938.

substance, and more recently Conn [20] produced diabetes in human beings by administration of pituitary adrenocorticotropic hormone. Persons with Addison's disease and diabetes are known to be notably sensitive to insulin; in such patients Thorn and Clinton [21] and Sprague and Kepler [22] observed that the use of Kendall's compound E (cortisone) resulted in increased glycosuria. Current theory [23] holds that increased blood sugar and glycosuria in the presence of increased adrenal cortex hormone are due to decreased glucose utilization rather than increased protein breakdown, as previously postulated by Long.[24] This hypothesis is in accord with the work of Cori,[25] who has shown in vitro that adrenal cortex extract in the presence of anterior pituitary extract inhibits the action of hexokinase, the enzyme which is essential for the conversion of glucose to glucose-6-phosphate as the initial step in the utilization of glucose by the human body.

All situations producing an increase in adrenal cortex function, such as surgical procedures, trauma, acute infectious processes, pituitary basophilism and adrenocortical carcinoma, necessitate a temporary increase in insulin dosage. When the underlying pathologic cause is corrected by appropriate treatment, the insulin requirement falls to its previous level. Patients in diabetic acidosis have a deficiency of insulin plus hyperactivity of the adrenal cortex (alarm reaction of Selye).[26]

INSULIN ALLERGY

Crystalline insulin is a protein molecule with a weight of 35,100, a value almost identical to that of egg albumin and Bence Jones protein.[27] It is well known for its antigenic properties and gives rise to two types of antibodies: reagin, which may be demonstrated by passive transfer and/or precipitation and is responsible for urticaria and other overt

20. Conn, J. W.; Louis, L. H., and Johnston, M. W.: Alleviation of Experimental Diabetes in Man by Administration of Reduced Glutathione (GSH): Metabolic Implications, Science **109**:279, 1949.

21. Thorn, G. W., and Clinton, M., Jr.: Metabolic Changes in a Patient with Addison's Disease Following Onset of Diabetes, J. Clin. Endocrinol. **3**:335, 1943.

22. Sprague, R. G.; Kepler, E. J.; Keating, F. R., Jr., and Power, M. H.: Effects of Synthetic 11-Dehydrocorticosterone (Compound A) in a Subject with Addison's Disease, Am. J. Med. **4**:175, 1948.

23. Knowlton, A. I.: The Adrenal Cortex (Combined Staff Clinics), Am. J. Med. **7**:105, 1949.

24. Long, C. N. H.; Katzin, B., and Fry, E. G.: The Adrenal Cortex and Carbohydrate Metabolism, Endocrinology **26**:309, 1940.

25. Cori, C. F.: Enzymatic Reactions in Carbohydrate Metabolism, in Harvey Lectures, 1945-1946, Baltimore, Williams & Wilkins Company, 1947, vol. 41, p. 253.

26. Selye, H.: The General Adaptation Syndrome and the Diseases of Adaptation, J. Clin. Endocrinol. **6**:117, 1946.

27. Sjogren, B., and Svedberg, T.: The Molecular Weight of Insulin, J. Am. Chem. Soc. **53**:2657, 1931.

allergic manifestations, and insulin-neutralizing antibodies, which neutralize the hypoglycemic effects of insulin but do not give rise to overt allergic manifestations, and which are probably responsible for a fairly high percentage of cases of true insulin resistance.

It may be noted in table 1 that 17 of these patients had manifestations of allergy to insulin: six had localized reactions at the site of injection; nine had generalized urticaria and rarely flushing and tachycardia, and two had eosinophilia. Impurities in the insulin preparation may have been responsible in some cases. Prausnitz-Küstner passive transfer antibodies were sought and found in four patients with allergic manifestations. In no patient in whom the passive transfer reaction was positive were insulin-neutralizing antibodies detected, and in only one patient in whom the precipitin reaction was positive were insulin-neutralizing antibodies detected. Of the 17 patients manifesting overt allergic phenomena, insulin-neutralizing antibodies were sought in 10 and detected in three. Five of the eight patients in whom insulin-neutralizing antibodies were detected had no allergic manifestations. These observations are in accord with those of Goldner and Ricketts,[28] who could find no fundamental relationship between insulin allergy and resistance to insulin. It appears that allergic reactions depend on the presence of an antibody or reagin which may be passively transferred to nonallergic persons or demonstrated by precipitation from the patient's serum. Administration of antihistaminic drugs, such as diphenhydramine (benadryl®) hydrochloride and tripelennamine (pyribenzamine®) hydrochloride, has been effective in controlling the overt manifestations of allergy to insulin.[29]

TRUE INSULIN RESISTANCE

Analysis of table 1 reveals the following points of interest:

Age.—Patients were found to vary in age from 12 to 79 years without remarkable preponderance of any age group.

Dosage.—The largest reported dose of insulin given in a 24 hour period was 5,820 units. The daily requirement varied considerably over a period of weeks or months and necessitated changes in dosage as resistance waxed and waned. Practically all patients responded adequately when sufficiently large doses of insulin were given.

Duration.—The shortest period of resistance in a patient who lived was 15 days; the longest was six years. Most patients were resistant to the hormone for a period varying from one month to one year.

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28. Goldner, M. G., and Ricketts, H. T.: Insulin Allergy: A Report of 8 Cases with Generalized Symptoms, J. Clin. Endocrinol. **2**:595, 1942.

29. Klein, S. P.: Insulin Allergy: Treatment with the Histamine Antagonists, Arch. Int. Med. **81**:316 (March) 1948.

Incidence of Allergy Associated with Resistance.—Thirty-three resistant patients showed no evidence of allergy; six displayed local reaction at the site of insulin injection; nine had generalized urticaria, and two had eosinophilia.

Insulin-Neutralizing Antibodies.—The serums of 26 patients were tested for circulating antibodies which protected mice or rabbits from the hypoglycemic effects of insulin.

In eight cases these antibodies were demonstrated; in one case the results were "equivocal," and in 17 cases no antibodies were detected.

Since insulin-neutralizing antibodies have been associated frequently with insulin resistance, further studies along these lines using more refined technics are needed. It is possible that circulating antibodies are missed in some cases, including the one in this communication, owing to failure to take into account large amounts of insulin already circulating in the patient's serum. Serum should be collected while circulating insulin is at a low level and the patient is actually in a state of resistance.

It is not necessary that a person have diabetes mellitus in order to become resistant to insulin. A nondiabetic schizophrenic patient undergoing shock therapy took 1,000 units in one intravenous injection with a resultant drop in the blood sugar from 115 to 80 mg. per 100 cc. Antibodies which protected mice from the convulsant effects of insulin were demonstrated in the patient's serum.[30] This suggests that there is no fundamental alteration in the carbohydrate cycle to account for insulin resistance.

Associated Diseases.—Five patients had pulmonary tuberculosis, three had hemochromatosis, three had rheumatoid arthritis and two had lipodystrophy. One patient each had acromegaly, chronic cholecystitis with cholelithiasis, chronic lymphatic leukemia, bilateral hydronephrosis, paresis, portal cirrhosis, congestive heart failure, dry gangrene of the foot, obscure destructive process of the head of the right femur, suspected Cushing's syndrome and arteriosclerotic heart disease. Two patients showed resistance shortly after having a thyroidectomy for thyrotoxicosis. In 24 patients there was no significant clinical evidence of associated disease.

Autopsy Observations.—Twenty-one patients of the 50 tabulated died, and postmortem examination was carried out in 17 instances. Four patients had fatty infiltration of the liver; two had hemochromatosis; two had pulmonary tuberculosis; two had portal cirrhosis; two had hyalinization of the islets of Langerhans; one had fibrosis of the pancreas; one had chronic cholecystitis; one had acute pancreatitis; one had arteriosclerotic heart disease, and one had peritonitis, hypernephroma and congestive heart failure.

30. Banting, F. G.; Franks, W. R., and Gairns, S.: Anti-Insulin Activity of Serum of Insulin Treated Schizophrenic Patient, Am. J. Psychiat. **115**:562, 1938.

By analysis of the disease and autopsy observations which have been reported in association with insulin resistance, it quickly becomes apparent that there have been no constantly associated pathological processes. Certainly those tabulated seem to be distributed by chance and appear to be wholly unrelated to the basic causes. Over 200 cases of hemochromatosis have been reported in the literature; most patients respond to insulin as would any other person with diabetes, and some are strongly sensitive to insulin.[31] Ten to 12 per cent of acromegalics have diabetes, and most respond to the injection of insulin as would any other diabetic.[32] Fatty infiltration of the liver frequently occurs in diabetics, especially in those who are not well regulated. The common practice in the current literature of attributing the phenomenon of insulin resistance to various pathological processes is not justified and should be discontinued.

The ultimate cause of insulin resistance remains obscure, although it now seems reasonably certain that circulating neutralizing antibodies result in a certain number of cases. At present we are not justified in concluding that all cases are due to a circulating antibody. Other possibilities to be considered are the inactivation of insulin by antibodies fixed in the tissues, particularly in the reticuloendothelial system,[33] and the presence in the body of a naturally occurring insulin antagonist. Buckley [34] demonstrated the fact that trypsin injected into rabbits will prevent the hypoglycemic effect of subsequently injected insulin. Mirsky and Broh-Kahn [35] recently demonstrated that aqueous extracts of liver, kidney and muscle quickly destroy insulin on incubation, but brain, whole blood and plasma do not. They were of the view that this is due to the presence of an anti-insulin substance which they called "insulinase." Sutherland and Cori [36] demonstrated that after intravenous injection of crystalline insulin into an intact animal an initial hyperglycemia occurs. They were of the opinion that this is due to the direct action on the liver of a "glycogenolytic factor" occurring in the insulin. Only negligible

31. Althausen, T. L., and Kerr, W. J.: Hemochromatosis: A Report of 3 Cases with Endocrine Disturbances and Notes on a Previously Reported Case; Discussion of Etiology, Endocrinology **17**:621, 1933. Sheldon, J. H.: Haemochromatosis, New York, Oxford University Press, 1935, p. 36.

32. Yater, W. M.: Acromegaly and Diabetes: Report of 6 Cases, Arch. Int. Med. **41**:883 (June) 1928.

33. Gordon, A. S.; Kleinberg, W., and Charipper, H. A.: Reticulo-Endothelial System and Hormone Refractoriness, J. Exper. Med. **70**:333, 1939.

34. Buckley, C. B.: The Effect of Subcutaneous Injections of Trypsin on the Blood Sugar and on Insulin Action, Brit. J. Exper. Path. **14**:57, 1933.

35. Mirsky, A.; Broh-Kahn, R. H.; Perisutti, G., and Brand, J.: The Inactivation of Insulin by Tissue Extracts: I. The Distribution and Properties of Insulin Inactivating Extracts (Insulinase), Arch. Biochem. **20**:1, 1949.

36. Sutherland, E. W., and Cori, C. F.: Influence of Insulin Preparations on Glycogenolysis in Liver Slices, J. Biol. Chem. **172**:737, 1948.

amounts of insulin are excreted, so that this factor can be of no importance as the cause of resistance.[37]

MANAGEMENT

The management of the insulin-resistant patient is not difficult. Causes of increased requirements as given in table 2 should be searched for and ruled out, and the patient's serum may be tested for insulin-neutralizing antibodies. He should be given an amount of insulin which is sufficient to control his glycosuria, regardless of how great that amount may be; there is probably no such thing as absolute refractoriness to the hormone. He should be cautioned to be on the lookout for insulin reactions and should carry sugar with him at all times, since sensitivity to insulin may return abruptly.

SUMMARY

A case of insulin-resistant diabetes associated with the autopsy observation of metastatic carcinoma of the pancreas and a critical review of the 50 cases of insulin resistance occurring in the literature form the basis of this report.

Insulin resistance is a state which requires 200 or more units of the hormone per day for longer than 48 hours for regulation of a nonacidotic person. It may occur at any age and may last a few days or several years. No fundamental relationship exists between resistance and allergy. There is no significant incidence of any associated disease or pathological process occurring in resistant patients. This indicates that the common practice in the literature of attributing resistance to various disease processes should be discontinued. The fact that it may occur in persons who do not have diabetes mellitus but are undergoing shock therapy suggests that an alteration of the carbohydrate cycle plays no role in its causation.

Insulin resistance should be differentiated from conditions causing fundamentally high requirements, as severe juvenile diabetes, and from disorders resulting in a temporary increase in insulin requirement: poor absorption from the injection site (insulin lipodystrophy, shock and

TABLE 3.—*Conditions Causing True Insulin Resistance*

I. Circulating insulin-neutralizing antibodies
II. Unknown
? Neutralization of insulin by fixed tissue antibodies
? Inactivation of insulin by substances naturally occurring in the body, such as trypsin, "insulinase" or "glycogenolytic factor"

37. Mirsky, I. A.; Podore, C. J.; Wachman, J., and Broh-Kahn, R. H.: The Urinary Excretion of Insulin by Normal and Diabetic Subjects, J. Clin. Investigation **27**:515, 1948.

congestive heart failure), hyperthyroidism, pheochromocytoma, surgical procedures, trauma, acute infectious processes, pituitary basophilism, adrenocortical carcinoma and diabetic acidosis.

The ultimate cause of insulin resistance is yet unknown, although it now seems reasonably certain that circulating insulin-neutralizing antibodies are the cause of a number of cases. Other possibilities to be clarified by future study are inactivation of insulin by antibodies fixed in the tissues, and the presence in excess in the body of a naturally occurring insulin antagonist, such as "insulinase," "glycogenolytic factor" or trypsin.

Management of insulin resistance is not difficult, since absolute refractoriness to the hormone probably does not occur and large doses nearly always have the desired effect of lowering the blood sugar. Therefore, these patients should be treated with amounts of insulin sufficient to control glycosuria, no matter how large that dose may be. They should be warned to watch for hypoglycemia, since sensitivity to the hormone may return abruptly.

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Progress in Internal Medicine

LIVER AND BILIARY TRACT

A Survey of Tests for Hepatic Function; the Use of the Hepatic Star in the Differential Diagnosis of Jaundice

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INTEREST in the functions of the liver has grown apace with the development of practical tests and the demonstration of the clinical usefulness of these tests in the study of patients with jaundice or other forms of hepatic disease. One of the first reviews of the subject was that of Rowntree, Marshall and Chesney in 1914.[1] My interest was initiated by a review published in 1925.[2] The field has expanded greatly since then. The table shows some of the major developments.

It is obvious that this field has expanded so greatly that, at present, it is too complex to be surveyed in detail in any one review. This presentation, therefore, is divided into two parts. The first will be a survey of recent developments concerning tests for hepatic function. These selected tests will be discussed in detail. In the second part, I shall present my views on the clinical use of these tests in the differential diagnosis of jaundice.

A SURVEY OF TESTS FOR HEPATIC FUNCTION

BILIRUBIN IN THE SERUM

Bilirubin is the excretory product formed by the breakdown of hemoglobin in the body. The various substances produced in the course of

From the Department of Medicine, New York University Post-Graduate Medical School and University Hospital, New York University-Bellevue Medical Center, New York. Part of the expense of this study was defrayed by a grant from the Difco Laboratories, Inc., Detroit.

1. Rowntree, L. G.; Marshall, E. K., Jr., and Chesney, A. M.: Studies in Liver Function, J. A. M. A. **63**:1533 (Oct. 31) 1914; Tr. A. Am. Physicians **29**:586, 1914.

2. Greene, C. H.; Snell, A. M., and Walters, W.: Diseases of the Liver: A Survey of Tests for Hepatic Function, Arch. Int. Med. **36**:248 (Aug.) 1925.

Classification of Liver Function Tests

I. The excretory functions of the liver
- A. Formation and secretion of bile
  - 1. Secretion of bile acids
    - a. Concentration of bile acids in blood
  - 2. Excretion of bile pigments
    - a. Bilirubin
      - 1′. Concentration of bilirubin in bile
      - 2′. Retention of bilirubin in blood
        - a′. Quantitative serum bilirubin
        - b′. Icterus index
        - c′. van den Bergh reaction
          - 1″. Bilirubinglobin (indirect reaction)
          - 2″. Bilirubin (direct reaction)
      - 3′. Excretion of bilirubin in urine
        - a′. Rosenbach nitric acid method
        - b′. Iodine ring test
        - c′. Methylene blue reaction
        - d′. Harrison spot test
        - e′. Barium chloride strip method
        - f′. Barium chloride tablet method
    - b. Urobilin and urobilinogen in urine
    - c. Porphyrin (urinary to fecal coproporphyrin ratio)
  - 3. Excretion of cholesterol (concentration of cholesterol in bile)
  - 4. Excretion of inorganic salts
    - a. Sodium chloride
    - b. Calcium
  - 5. Excretion of water
- B. Excretory loading tests
  - 1. Bilirubin
    - a. Bilirubin excretion test
  - 2. Dyes
    - a. Phenoltetrachlorphthalein test
    - b. Rose bengal test
    - c. Azorubin S test
    - d. Sulfobromophthalein test
    - e. Phenoltetraiodophthalein test

II. The metabolic functions of the liver
- A. Carbohydrate metabolism
  - 1. Fasting blood sugar
  - 2. Galactose tolerance test
    - a. Oral
    - b. Intravenous
  - 3. Dextrose tolerance test
  - 4. Levulose tolerance test
  - 5. Utilization of *d*-lactic acid
- B. Protein metabolism
  - 1. Concentration of amino acids in blood
  - 2. Concentration of urea in blood
  - 3. Concentration of ammonia in blood
  - 4. Concentration of uric acid in blood
  - 5. Concentration of guanidine in blood
  - 6. Tyrosine tolerance test
  - 7. Methionine clearance test
- C. Fat metabolism
  - 1. Concentration of cholesterol in blood
    - a. Total cholesterol
    - b. Cholesterol partition (ratio of esters to total cholesterol
  - 2. Fat tolerance tests
    - a. Oral
    - b. Intravenous
  - 3. Lipid partition in blood
- D. Blood-forming functions
  - 1. Erythrocytes (macrocytic hyperchromic anemia)
  - 2. Plasma proteins
    - a. Fibrinogen
    - b. Albumin and globulins
      - 1′. Concentration of serum albumin
      - 2′. Takata-Ara reaction
      - 3′. Formol-gel reaction
      - 4′. Weltmann coagulation band
      - 5′. Magnesium chloride test
      - 6′. Colloidal gold reaction
      - 7′. Cephalin cholesterol flocculation
      - 8′. Thymol turbidity
      - 9′. Thymol flocculation
      - 10′. Colloidal red precipitation
      - 11′. Zinc sulfate precipitation
      - 12′. Gamma globulin determination
      - 13′. Electrophoretic analysis
    - c. Prothrombin
      - 1′. Prothrombin time
      - 2′. Response of prothrombin time to vitamin K administration
- E. Detoxification function
  - 1. Hippuric acid synthesis
    - a. Oral
    - b. Intravenous
  - 2. Cinchophen test
  - 3. Glucuronic acid test
- F. Phosphatase excretion
  - 1. Serum alkaline phosphatase

this breakdown and the methods for their recognition have been discussed in detail by Watson [3] and need not be reiterated here. The color reaction popularized by van den Bergh [4] was the first to show a difference between the bilirubin normally present in the serum, which gives an indirect van den Bergh reaction, and that present in the bile, which gives a direct van den Bergh reaction. In cases of uncomplicated hemolytic jaundice the van den Bergh reaction of the serum is indirect, but in cases of jaundice due to hepatitis or biliary obstruction (regurgitant jaundice, according to the classification of Rich [5]) the van den Bergh reaction is direct. While a direct van den Bergh reaction cannot be used to differentiate between the hepatic and the obstructive types of jaundice, it was soon recognized that at the onset of either type of jaundice the van den Bergh reaction becomes direct before the serum bilirubin is elevated significantly. Likewise, when jaundice is clearing, the van den Bergh reaction remains direct for a time after the serum bilirubin has returned to normal.

Several investigators tried to determine the two bilirubin compounds separately.[6] Most commonly, this was done by extracting the direct-reacting pigment with chloroform. Interest in this problem stemmed from the thought that such a separation would help in determining the relative importance of the processes of retention and regurgitation in the pathogenesis of any individual case of jaundice. It soon became apparent

3. Watson, C. J.: Some Newer Concepts of the Natural Derivatives of Hemoglobin, Blood **1**:99, 1946.

4. Van den Bergh, A. A. H., in collaboration with J. Snapper and J. Muller: Der Gallenfarbstoff im Blute, Leiden, S. C. Van Doesburgh, 1918, p. 111.

5. Rich, A. R.: The Pathogenesis of the Forms of Jaundice, Bull. Johns Hopkins Hosp. **47**:338, 1930.

6. Varela, B., and Esculies, J.; Nouvelle méthode pour la séparation et le dosage des deux bilirubines (directes et indirectes) du sérum sanguin, Compt. rend. Soc. de biol. **107**:884, 1931. Varela, B.; Recarte, P., and Esculies, J.: Méthode simplifiée pour la séparation et le dosage isolé des deux bilirubines, directe et indirecte, du sérum sanguin dans les ictères, ibid. **108**:1009, 1931. Kerpolla, W.: Extraction Method for the Quantitative Determination of Bilirubin in Different Body-Fluids, Acta. med. Scandinav., 1932, supp. 50, p. 277. Varela-Fuentes, B., and Recarte, P.: Nouvelle technique simplifiée pour la dosage séparé des deux bilirubines, directe et indirecte, des sérums ictériques, Compt. rend. Soc. de biol. **116**:1193, 1934. Bengolea, A. J.; Velasco Suárez, C., and Raices, A. E.: El dosaje de la bilirrubina directa e indirecta en el suergo sanguíneo: Su importancia en cirurgía hepato-biliar, Prensa méd. argent. **23**:85, 1936. Greene, C. H.; Plotz, M., and Localio, S. A.: Liver and Biliary Tract: A Review for 1937, Arch. Int. Med. **61**:655 (April) 1938. Sepulveda, B., and Osterberg, A. E.: Modified Technic for Determination of Serum Bilirubin: Preliminary Report of Its Clinical Use, ibid. **72**:372 (Sept.) 1943. Schaffner, F.; Popper, H., and Steigmann, F.: The Significance of Bilirubin Partition in Hepato-Biliary Diseases, Am. J. M. Sc. **219**:307, 1950.

that in most cases of jaundice this method showed an increase of both types of bilirubin. Furthermore, the chloroform extraction was a far from satisfactory method of separation.

Ducci and Watson [7] have modified the procedure significantly. They add diazo reagent to the blood serum and read the intensity of the diazo reaction in a photoelectric colorimeter at the end of 60 seconds precisely. The total bilirubin is then determined by the method of Malloy and Evelyn.[8] By this technic the 60-second reading is a measure of the direct-reacting bilirubin, while the difference between that and the total is a measure of the indirect-reacting bilirubin. The results of this test, then, have the same significance as the direct van den Bergh reaction, but the test has the great advantage of being a quantitative rather than a qualitative one.

BILIRUBIN IN THE URINE

In cases of hepatitis bile pigments appear in the urine before the skin becomes yellow. In some cases only the urine and not the skin is discolored. These observations, originally reported by Budd [9] over a century ago, have been reemphasized by the recent studies of Neefe and his associates [10] on acute hepatitis and of Myers [11] on exposure to tetrachlorethane. Their work has led to a reinvestigation of tests for bilirubin in the urine. Myers advocated the methylene blue test of Franke,[12] while Stokes [13] and his associates preferred the modification of Fellinger and Menkes,[14] which permits a semiquantitative study of the pigment in the urine.

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7. Ducci, H., and Watson, C. J.: The Quantitative Determination of the Serum Bilirubin with Special Reference to the Prompt-Reacting and the Chloroform Soluble Types, J. Lab. & Clin. Med. **30**:293, 1945. Ducci, H., and Roeschmann, W.: Bilirrubinemia directa pronta y su importancia clínica, Rev. méd. de Chile **77**: 192, 1949.

8. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin with Photoelectric Colorimeter, J. Biol. Chem. **119**:481, 1937.

9. Budd, G.: On Diseases of the Liver, ed. 2, London, J. & A. Churchill, Ltd., 1852, p. 459.

10. Neefe, J. R.; Stokes, J., Jr.; Reinhold, J. G., and Lukens, F. D. W.: Hepatitis Due to the Injection of Homologous Blood Products in Human Volunteers, J. Clin. Investigation **23**:836, 1944.

11. Myers, C. P.: Use of Methylene Blue in Testing for Bilirubin in the Urine, J. Indust. Hyg. & Toxicol. **27**:52, 1945.

12. Franke, K.: Methylenblau, ein einfaches sehr empfindliches Reagens zum Nachweis von Bilirubin, Med. Klin. **27**:94, 1931.

13. Stokes, J., Jr., and Neefe, J. R.: The Prevention and Attenuation of Infectious Hepatitis by Gamma Globulin, J. A. M. A. **127**:144 (Jan. 20) 1945. Gellis, S. S., and Stokes, J., Jr.: The Methylene Blue Test in Infectious (Epidemic) Hepatitis, ibid. **128**:782 (July 14) 1945.

14. Fellinger, K., and Menkes, K.: Ueber quantitative Bilirubinbestimmungen im Harn mit der Methylenblaumethode, Wien. klin. Wchnschr. **46**:133, 1933.

The methylene blue test is not specific for bilirubin; rather it is a measure of the intensity of the yellow of the urine. When used to measure the day to day changes in the color of the urine in patients with hepatitis, the test is fairly satisfactory. A considerable number of workers [15] has pointed out that too many other substances will color the urine yellow, and so give a positive reaction with methylene blue, to permit its use on random specimens of urine.

Foord and Baisinger [16] studied various tests and reported that the Harrison spot test [17] was as sensitive and specific a test of bilirubin as any. For the Harrison test, barium chloride solution is added to the urine and the precipitate of barium and bilirubin is collected by filtering. Addition of a drop of Fouchet's reagent produces a characteristic green color if bilirubin is present. When mass surveys are undertaken, the filtration of the urine takes some time. Hawkinson, Watson and Turner,[18] therefore, have sought to simplify the test further by impregnating strips of thick filter paper with barium chloride. Such a strip is suspended in the urine specimen for one minute, then it is removed and a drop of Fouchet's reagent added.

Franklin has modified this test [19] by using large tablets of plaster of paris impregnated with barium chloride. Ten drops (0.5 ml.) of urine are dropped slowly on the tablet so that the urine filters through without overflow. If bilirubin is present, it will be adsorbed on the surface of

15. Methylene Blue Test for Urinary Bilirubin, editorial, J. A. M. A. **127:** 1058 (April 21) 1945. Watson, J.; Meads, M., and Castle, W. B.: Tests for Urinary Bilirubin, Correspondence, ibid. **128:**308 (May 26) 1945. Figge, F. H. J.: The Green Color of a Methylene Blue-Bilirubin Mixture, Correspondence, ibid. **128:**613 (June 23) 1945. Gellis, S. S.; Neefe, J. R.; Reinhold, J. G., and Stokes, J., Jr.: Methylene Blue Test for Bilirubin in the Urine, Correspondence, ibid. **128:** 826 (July 14) 1945. Haffke, O. W.: Tests for Infectious Hepatitis, Correspondence, ibid. **129:**647 (Oct. 27) 1945. Stokes, G. D.; Gambill, E. E., and Osterberg, A. R.: The Methylene Blue Test for Bilirubinuria: Clinical and Spectrophotometric Observations, J. Lab. & Clin. Med. **39:**924, 1946. Lipp, W. F., and Lenzner, A. R.: Observation on the Methylene Blue Test for Bile Pigment in Urine, Gastroenterology **8:**154, 1947. Holt, C. J.: The Value of the Methylene Blue Test in the Detection of Bilirubin, New England J. Med. **237:**580, 1947.

16. Foord, A. G., and Baisinger, C. F.: Comparison of Tests for Bilirubin in Urine, Am. J. Clin. Path. **10:**238, 1940.

17. Harrison, G. A.: Chemical Methods in Clinical Medicine: Their Application and Interpretation with the Technique of the Simple Tests, ed. 2, London, J. & A. Churchill, Ltd., 1937.

18. Hawkinson, V.; Watson, C. J., and Turner, R. H.: A Modification of Harrison's Test for Bilirubin in the Urine Especially Suited for Mass and Serial Usage, J. A. M. A. **129:**514 (Oct. 13) 1945. Watson, C. J., and Hawkinson, V.: Semi-Quantitative Estimation of Bilirubin in Urine by Means of Barium-Strip Modification of Harrison's Test, J. Lab. & Clin. Med. **31:**914, 1946.

19. Franklin, M.: A New Tablet Test for Urinary Bilirubin, J. Lab. & Clin. Med. **34:**1145, 1949.

the tablet and can be demonstrated by adding 1 to 2 drops of Fouchet's reagent. Both the barium chloride tablet and the barium chloride strip tests simplify the technic of the Harrison test for bilirubin in the urine. Since the amount of urine used is limited, they may be expected to be slightly less sensitive than the Harrison test.

EXCRETION OF DYES AS TESTS OF HEPATIC FUNCTION

Various materials have been employed in excretory loading tests of hepatic function. Phenoltetrachlorphthalein was the first dye used in this fashion. It was introduced into clinical study by Rowntree, Hurwitz and Bloomfield,[20] who determined the amount of dye recovered in the stools. McNeil,[21] Piersol and Bockus [22] and others [23] substituted the passage of a duodenal tube and reported on the time of appearance of the dye in the duodenal drip and the amount recovered during a two-hour period of drainage. Rosenthal [24] suggested determining the rate of removal of the dye from the blood stream as the most satisfactory clinical test of hepatic disturbance. This modification was quickly accepted,[25]

20. Rowntree, L. G.; Hurwitz, S. H., and Bloomfield, A. L.: An Experimental and Clinical Study of the Value of Phenoltetrachlorphthalein as a Test for Hepatic Function, Bull. Johns Hopkins Hosp. **24**:327, 1913; Der Wert des Phenoltetrachlorphthalein für die Funktionsprüfung der Leber, Arch. f. Verdauungskr. **19**:751, 1913.

21. McNeil, H. L.: The Quantitative Estimation of Phenoltetrachlorphthalein Excreted in the Fresh Bile in Disease of the Liver, J. Lab. & Clin. Med. **1**:822, 1915.

22. Piersol, G. M., and Bockus, H. L.: The Value of Phenoltetrachlorphthalein in Estimating Liver Function, Tr. A. Am. Physicians **37**:433, 1922; Arch. Int. Med. **31**:623 (May) 1923; Comparative Studies in Liver Function by Some of the Later Methods, J. A. M. A. **83**:1043 (Oct. 4) 1924.

23. Kahn, M.: Phenoltetrachlorphthalein Estimation in the Duodenal Contents, J. A. M. A. **77**:41 (July 2) 1921. Aaron, A. H.; Beck, E. C., and Schneider, H. C.: The Phenoltetrachlorphthalein Test for Liver Function, ibid. **77**:1631 (Nov. 19) 1921. Friedenwald, J., and Gantt, W. H.: Some Observations on the Phenoltetrachlorphthalein Test as a Means of Determining Liver Function, Am. J. M. Sc. **166**:519, 1923.

24. Rosenthal, S. M.: An Improved Method for Using Phenoltetrachlorphthalein as a Liver Function Test, J. Pharmacol. & Exper. Therap. **19**:385, 1922; Testing Liver Function with Phenoltetrachlorphthalein, Bull. Johns Hopkins Hosp. **33**:432, 1922; A New Method for Testing Liver Function with Phenoltetrachlorphthalein: III. Clinical Report, J. A. M. A. **79**:2151 (Dec. 23) 1922; IV. The Relation of Impaired Function to the Amount of Normal Liver Tissue, J. Pharmocol. & Exper. Therap. **23**:385, 1924; The Phenoltetrachlorphthalein Test for Hepatic Function: Recent Studies with the Author's Method, J. A. M. A. **83**: 1049 (Oct. 4) 1924.

25. Rosenthal, H. H., and Schneiders, E. F.: Improved Phenoltetrachlorphthalein Test for Liver Function in Pregnancy and Its Toxemias, J. A. M. A. **80**: 743 (March 17) 1923. Greenbaum, S. S., and Brown, H.: The Phenoltetrachlorphthalein Liver Test in Cases of Acute and Chronic Syphilis Under Treatment in Various Skin Diseases, ibid. **82**:88 (Jan. 12) 1924.

and an extensive literature dealing with the clinical uses of the phenoltetrachlorphthalein test soon developed.[26]

Other dyes, such as rose bengal [27] and azorubin S,[28] have been introduced as substitutes for phenoltetrachlorphthalein. Because they considered dyes foreign to the animal economy, von Bergmann [29] and Eilbott [30] substituted a substance normally manufactured in the body,

26. Snell, A. M.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: II. A Comparative Study of Certain Tests for Hepatic Function in Experimental Jaundice, Arch. Int. Med. **36**:273 (Aug.) 1925. Greene, C. H.; McVicar, C. S.; Rowntree, L. G., and Walters, W.: Diseases of the Liver: III. A Comparative Study of Certain Tests for Hepatic Function in Patients with Obstructive Jaundice, ibid. **36**:418 (Sept.) 1925. Greene, C. H.; McVicar, C. S.; Walters, W., and Rowntree, L. G.: Diseases of the Liver: IV. Functional Tests in Cases of Carcinoma of the Liver and Biliary Tract, ibid. **36**:542 (Oct.) 1925. Greene, C. H., and Conner, H. M.: Diseases of the Liver: V. A Comparative Study of Tests for Hepatic Function in Certain Diseases of the Hematopoietic System, ibid. **38**:167 (Aug.) 1926. Greene, C. H.; McVicar, C. S.; Snell, A. M., and Rowntree, L. G.: Diseases of the Liver: VI. A Comparative Study of Certain Tests for Hepatic Function in Cases of Cirrhosis of the Liver, ibid. **40**:159 (Aug.) 1927.

27. Delprat, G. D.; Epstein, N. N., and Kerr, W. J.: A New Liver Function Test: Elimination of Rose Bengal When Injected into Circulation of Human Subjects, Arch. Int. Med. **34**:533 (Oct.) 1924. Epstein, N. N.; Delprat, G. D., and Kerr, W. J.: The Rose Bengal Test for Liver Function, J. A. M. A. **88**:1619 (May 21) 1927. Delprat, G. D., and Stowe, W. P.: The Rose Bengal Test for Liver Function, J. Lab. & Clin. Med. **16**:923, 1931. Stowe, W. P.; Delprat, G. D., and Weeks, A.: The Rose Bengal Test for Liver Function, Am. J. Clin. Path. **3**: 55, 1933. Althausen, T. L.; Biskind, G. R., and Kerr, W. J.: The Rose Bengal Test of Hepatic Function: A Spectroscopic Method, J. Lab. & Clin. Med. **18**:954, 1933. Radhakrishna Roa, M. V.: The Clinical Value of the Rose Bengal Test for the Determination of the Total Functional Capacity of the Liver, Indian J. M. Research **20**:1009, 1933. Monroe, L., and Hopper, J., Jr.: A Comparison of the Bromsulfalein and Rose Bengal Tests, J. Lab. & Clin. Med. **34**:246, 1949.

28. Tada, Y., and Nakashima, K.: A New Dye Test of Liver and Biliary Tract Function, with Especial Reference to Its Clinical Use, J. A. M. A. **83**:1292 (Oct. 25) 1924; Jap. J. Gastroenterol. (supp.) **6**:386, 1934. Fenstermann, R.: Die Funktionsprüfung der Leber mit Azorubin S, München. med. Wchnschr. **73**: 859, 1926. Zinny, M.: El estudio de la función hepática, en especial por la azorrubina, Prensa méd. argent. **13**:665, 1926. Lebedow, A. J.: Die chromocholoskopische Azorubinprobe, Arch. f. Verdauungskr. **47**:131, 1930. Nakashima, K.: Chromocholoscopy, Jap. J. Gastroenterol. (supp.) **6**:396, 1934. Weiss, S.: Diseases of the Liver, Gall Bladder, Ducts and Pancreas: Their Diagnosis and Treatment, New York, Paul G. Hoeber, Inc., 1935. Eppinger, H.: Die Leberkrankheiten: Allgemeine und spezielle Pathologie und Therapie der Leber, Vienna, Springer-Verlag, 1937, pp. 179-180. Rosenberg, D. H., and Soskin, S.: The Azorubin S Test of Liver Function: An Evaluation with a Comparative Study of Bromsulphalein and Hippuric Acid Test, Ann. Int. Med. **13**:1644, 1940.

29. von Bergmann, G.: Zur funktionellen Pathologie der Leber insbesondere der Alkohol-Aetiologie der Cirrhose, Klin. Wchnschr. **6**:766, 1927.

30. Eilbott, W.; Funktionsprüfung der Leber mittels Bilirubinbelastung, Ztschr. f. klin. Med. **106**:529, 1927.

namely, bilirubin. A solution containing 1 mg. per kilogram of body weight was injected intravenously, and blood specimens were taken five minutes and four hours after the injection. A retention of more than 5 per cent of the injected pigment at the end of four hours was considered abnormal. The proponents of this test are enthusiastic about it.[31] In my hands bilirubin was expensive and difficult to procure. Making a fresh solution for each test was troublesome. For these reasons, the bilirubin test has generally been supplanted by the sulfobromophthalein (bromsulphalein®) sodium excretion test.

Sulfobromophthalein was introduced by Rosenthal and White.[32] Because the dye is more soluble, and its solution more stable, much less irritating to the tissue and rarely a cause of venous thrombosis, it has generally become the substance of choice in an excretory test of hepatic function.

There have been various modifications of Rosenthal's original technic. Greene[33] increased the dose of sulfobromophthalein from 2 to 5 mg. per kilogram of body weight, which greatly increased the sensitivity of the test. He eliminated the five-minute specimen of blood and took a specimen at 30 minutes. If this specimen showed retention, a second specimen was taken 60 minutes after the injection.

MacDonald[34] used a dose of 5 mg. of dye per kilogram of body weight but determined the curve of excretion by taking blood samples

31. (*a*) Jankelson, I. P., and Gargill, S. R.: Bilirubin Liver Function Test: I. Modification of Method, New England J. Med. **204**:547, 1931. (*b*) Harrop, G. A., and Barron, E. S. G.: The Excretion of Intravenously Injected Bilirubin as a Test of Liver Function, J. Clin. Investigation **9**:577, 1931. (*c*) Soffer, L. J.: Bilirubin Excretion as a Test for Liver Function During Normal Pregnancy, Bull. Johns Hopkins Hosp. **52**:365, 1933. (*d*) Soffer, L. J., and Paulson, M.: Residual Hepatic Damage in Catarrhal Jaundice as Determined by the Bilirubin Excretion Test, Arch. Int. Med. **53**:809 (June) 1934. (*e*) Soffer, L. J.: Present Day Status of Liver Function Tests, Medicine **14**:185, 1935. (*f*) Strasser, U.: Zur Bewertung der Bilirubinbelastungprobe nach v. Bergmann-Eilbott, Wien. Arch. f. inn. Med. **31**:267, 1937. (*g*) Kornberg, A.: Latent Liver Disease in Persons Recovered from Catarrhal Jaundice and in Otherwise Normal Medical Students as Revealed by the Bilirubin Excretion Test, J. Clin. Investigation **21**:299, 1942.

32. Rosenthal, S. M., and White, E. C.: Clinical Application of the Bromsulphalein Test for Hepatic Function, J. A. M. A. **84**:1112 (April 11) 1925; Studies in Hepatic Function: VI. The Pharmacological Behavior of Certain Phthalein Dyes; The Value of Selected Phthalein Compounds in the Estimation of Hepatic Function, J. Pharmacol & Exper. Therap. **24**:265, 1924.

33. O'Leary, P. A.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: VIII. The Various Types of Syphilis of the Liver with Reference to Tests of Hepatic Function, Arch. Int. Med. **44**:155 (Aug.) 1929.

34. MacDonald, D.: Some Observations of the Disappearance of Bromsulphalein Dye from the Blood: Its Relation to Liver Function, Canad. M. A. J. **39**: 556, 1938; A Practical Clinical Test for Liver Reserve, Surg., Gynec. & Obst. **69**: 70, 1939.

every two minutes. Mateer [35] made similar studies and then selected 45 minutes as the best time at which to take a single specimen for routine use. It is obvious that the sensitivity of the sulfobromophthalein test can be increased progressively by increasing the dose and, thus, the load on the liver. On the other hand, it is equally obvious that the greater the sensitivity of the test the greater the effect of various physiological factors, such as the nutritional status of the patient and the degree of anemia or of changes in the circulation. It is my personal opinion that the present technic, with a dose of 5 mg. of sulfobromophthalein per kilogram of body weight and either a 30 or 45 minute specimen of blood, probably represents the most satisfactory compromise among the various factors, such as sensitivity of test, simplicity of technic and sharpness of clinical differentiation.

When the various dyes were first introduced for the study of hepatic function, there was considerable discussion concerning the mechanism by which they were removed from the blood and the relative importance of the hepatic cells and of the reticuloendothelial system in such removal.[36] Various studies of this problem were made using india ink, trypan blue or the like to produce a reticuloendothelial block. Such studies were indirect. I have noted that the injection of sulfobromophthalein produces

35. Mateer, J. G.; Baltz, J. I.; Marion, D. F.; Hollands, R. A., and Yagle, E. M.: A Comparative Evaluation of the Newer Liver Function Tests, Am. J. Digest. Dis. **9**:13, 1942. Mateer, J. G.; Baltz, J. I.; Marion, D. F., and MacMillan, J. M.: Liver Function Tests: A General Evaluation of Liver Function Tests, and an Appraisal of the Comparative Sensitivity and Reliability of the Newer Tests, with Particular Emphasis on the Cephalin-Cholesterol Flocculation Test, the Intravenous Hippuric Acid Test and an Improved Bromsulphalein Test with a New Normal Standard, J. A. M. A. **121**:723 (March 6) 1943. Mateer, J. G.; Baltz, J. I.; Comanduras, P. D.; Steele, H. H., and Brouwer, S. W.: Further Advances in Liver Function Tests, and the Value of a Therapeutic Test in Facilitating the Earlier Diagnosis and Treatment of Liver Impairment, Gastroenterology **8**: 52, 1947. Mateer, J. G.; Baltz, J. I.; Steele, H. H.; Brouwer, S. W., and Colvert, J. R.: Chronic Subclinical Impairment of the Liver: Early Diagnosis and Treatment; Further Improvement and Evaluation of Certain Liver Function Tests, J. A. M. A. **133**:909 (March 29) 1947.

36. Mills, M. A., and Dragstedt, C. A.: Bromsulphalein Dye Retention Test as a Measure of Functional Activity of the Reticulo-Endothelial System, Proc. Soc. Exper. Biol. & Med. **34**:228, 1936; Removal of Intravenously Injected Bromsulphalein from the Blood Stream of the Dog, Arch. Int. Med. **62**:216 (Aug.) 1938. Cantarow, A., and Wirts, C. W., Jr.: Excretion of Bromsulfalein in the Bile, Proc. Soc. Exper. Biol. & Med. **47**:252 1941. Wirts, C. W., Jr., and Cantarow, A.: A Study of the Excretion of Bromsulphalein in the Bile, Am. J. Digest. Dis. **9**:101, 1942. Cohn, C.; Levine, R., and Streicher, D.: The Rate of Removal of Intravenously Injected Bromsulphalein by the Liver and Extra-Hepatic Tissues of the Dog, Am. J. Physiol. **150**:229, 1947. Moses, C.; Critchfield, F. H., and Thomas, T. B.: The Importance of the Rate of Dye Removal in the Bromsulfalein Test of Liver Function, J. Lab. & Clin. Med. **33**:448, 1948.

a transitory increase in the serum bilirubin. This could be taken as evidence of blocking of the hepatic cells by the dye.

More direct evidence on secretion by the liver has been supplied by Grafflin,[37] who used fluorescein, and by Mendeloff,[38] who used rose bengal. Both these dyes show a characteristic fluorescence and can be visualized in the hepatic cells by fluorescent microscopy. The two sets of observations indicated that the dyes were rapidly picked up by the polygonal cells of the liver and passed through to the bile capillaries. The dye was not visualized in the Kupffer cells. These observations are strong evidence that the excretion of the dyes mentioned, and by analogy of sulfobromophthalein, is normally a function of the parenchymal cells of the liver.

At present, the sulfobromophthalein test is generally accepted as one of the simplest and most sensitive tests of hepatic function. In my experience it is one of the earliest to show changes at the inception of acute hepatitis. It reveals changes early in cases of cardiac decompensation with beginning passive congestion. It is one of the tests most sensitive to the changes in metastatic infiltration of the liver. It can be used in cases of hepatitis to follow the course of the jaundice, but in most cases the serum bilirubin level or icterus index provides similar information with less difficulty. Jaundice does not contraindicate the use of the test, but it is of less value in the differential diagnosis of jaundice because one cannot be sure how much of the dye retention is due to injury to the hepatic cells and how much is due to biliary obstruction.

For the most part, sulfobromophthalein has been a satisfactory dye for use in testing hepatic function. The solution in ampuls is stable and can be given intravenously without special apparatus. Local reactions following improper injection of the dye into extravascular or paravascular tissues occur but can be avoided by correct technic. Vascular thrombosis or phlebitis is infrequent.[39] Generalized toxic reactions, with headache, faintness and chills, occur and in my experience are associated with particular lots of ampuls. Such reactions have not been observed for several years. Recently, Morey and his associates [40] have reported

37. Grafflin, A. L.: The Excretion of Fluorescein by the Liver Under Normal and Abnormal Conditions, Observed in Vivo with the Fluorescence Microscope, Am. J. Anat. **81**:63, 1947; Studies of Fluorescein Excretion by the Liver Under Normal and Abnormal Conditions, in the Seventh Conference on Liver Injury, New York, Josiah Macy, Jr., Foundation, 1948.

38. Mendeloff, A. I.: Fluorescence of Intravenously Administered Rose Bengal Appears Only in Hepatic Polygonal Cells, Proc. Soc. Exper. Biol. & Med. **70**:556, 1949.

39. Stempien, S. J., and Rosenquist, R. C.: Bromsulfalein Reactions: Clinical and Experimental Study, Gastroenterology **12**:978, 1949.

40. Morey, G.; Gabuzda, G. J., and Scudamore, H. H.: Sensitization to Bromsulphalein (Phenoltetrabromphthalein-Disodium Sulphonate), Gastroenterology **13**: 246, 1949.

allergic reactions in three patients following the use of serial tests. This is an unusual type of reaction but should be watched for, as more and more patients are being subjected to repeated tests.

Ingelfinger and his associates [41] have used sulfobromophthalein in determining the estimated blood flow through the liver. In their procedure the dye is injected at a constant rate, the hepatic veins are catheterized and the estimated blood flow is calculated from the rate of injection and the amount of dye removed from each 100 ml. of plasma during its passage through the liver. This is an important line of investigation, but the studies have not yet advanced to the point where they can be used as a routine clinical test. A discussion of this method is, therefore, outside the scope of this review.

CARBOHYDRATE METABOLISM

The investigation of the role of the liver in carbohydrate metabolism dates from the pioneer studies of Claude Bernard. Clinical tests of hepatic function have been concerned primarily with levulose and galactose tolerances. The reserve of the liver is so great that these tests ordinarily do not show changes until severe damage has been produced. The time factor, among others, is important in determining the stage of the disease and in interpreting the results of the tests. Since this subject has been discussed in detail in such reviews as those of Soffer,[31e] Lichtman [42] and Tumen,[43] it need not be gone into here. While these tests have been important in the historical development of knowledge of hepatic function tests, I have largely replaced them in my clinical practice with several of the more sensitive procedures.

41. Bradley, S. E.; Ingelfinger, F. J.; Bradley, G. P., and Curry, J. J.: The Estimation of Hepatic Blood Flow in Man, J. Clin. Investigation **24**:890, 1945. Bradley, S. E.: Variations in Hepatic Blood Flow in Man During Health and Disease, New England J. Med. **240**:456, 1949. Culbertson, J. W.; Wilkins, R. W.; Ingelfinger, F. J., and Bradley, S. E.: The Effect of the Upright Posture upon Hepatic Blood Flow in Normal and Hypertensive Human Subjects, J. Clin. Investigation **26**:1178, 1947. Ingelfinger, F. J.; Bradley, S. E.; Mendeloff, A. I., and Kramer, P.: Studies with Bromsulfalein: I. Its Disappearance from the Blood After a Single Intravenous Injection, Gastroenterology **11**:646, 1948. Bradley, S. E.; Ingelfinger, F. J.; Gross, A. E., and Bradley, G. P.: Estimated Hepatic Blood Flow and Hepatic Venous Oxygen Content in Cirrhosis of the Liver, Proc. Soc. Exper. Biol. & Med. **67**:206, 1948. Mendeloff, A. I.; Kramer, P.; Ingelfinger, F. J., and Bradley, S. E.: Studies with Bromsulfalein: II. Factors Altering Its Disappearance from the Blood After a Single Intravenous Injection, Gastroenterology **13**:222, 1949.

42. Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bile Ducts, Philadelphia, Lea & Febiger, 1942.

43. Tumen, H. J.: Diagnostic Laboratory Methods Applicable to the Study of Liver Function and Disease: Liver Function Tests; X-Ray Study of the Liver and Aspiration Biopsy, in Bockus, H. L., and others: Gastro-Enterology, Philadelphia, W. B. Saunders Company, 1946, vol. 3, pp. 62-102.

CHANGES IN THE SERUM PROTEINS IN HEPATIC DISEASE

Attention has been focused on a number of flocculation tests which show qualitative changes in the composition and colloidal stability of the blood serum and which make up in simplicity and ease of analysis what they lack in quantitative accuracy. These tests fall into two groups so far as their diagnostic use in the study of hepatic disease is concerned.

Flocculation Tests.—The first group comprises the formol-gel test,[44] the dilution test of Naumann,[45] the Takata-Ara test,[46] the Hayem test,[47] the magnesium chloride test of Bauer [48] and the Weltmann test.[49] These

44. Cantarow, A., and Trumper, M.: Clinical Biochemistry, ed. 4, Philadelphia, W. B. Saunders Company, 1949.

45. Naumann, H. R.: Saturation of Serum with CO_2: A Simple Test for Hyperglobulinemia, Proc. Soc. Exper. Biol. & Med. **39**:378, 1938.

46. Levinson, S. A., and Mac Fate, R. P.: Clinical Laboratory Diagnosis, ed. 3, Philadelphia, Lea & Febiger, 1946. Gradwohl, R. B. H.: Clinical Laboratory Methods and Diagnosis: A Textbook on Laboratory Procedures with Their Interpretation, ed. 3, St. Louis, C. V. Mosby Company, 1943.

47. Gros, W.: Eine neue, einfache Flockungsreaktion mit Hayemscher Lösung, Klin. Wchnschr. **18**:781, 1939; Weitere Erfahrungen über die Flockungsreaktion mit Hayemscher Lösung, ibid. **19**:1930, 1940. Stolte, J. B.: Mercurous Chloride Titration of Serum: Simple Reaction to Substitute for Takata Test, Nederl. tijdschr. v. geneesk. **84**:4887, 1940. Vischer, A.: Die Bedeutung der Serumreaktion mit Hayemscher Lösung im Vergleich zur Takatareaktion, Schweiz. med. Wchnschr. **71**:659, 1941. Gros, W.: Bedeutung der Flockungsreaktion mit Hayemscher Lösung im klinischen Verlaufsbild des Parenchymikterus, Klin. Wchnschr. **21**:877, 1942; Titration des Serums mit Hayemscher Lösung als differentialdiagnostische Untersuchungsmethode zwischen einem parenchymatösen und mechanischen Ikterus, ibid. **21**:969, 1942. Hammersten, G., and Stahle, I.: Observations on Jaundice, with Special Consideration of Epidemic Hepatitis, Nord. med. (Hygiea) **20**:2427, 1943. Hafström, T. G.: in discussion on Hannfors: Experience with Flocculation Reaction According to Gros, ibid. **23**:1365, 1944. Emmrich, R.: Die Flockungsreaktion im Verlaufe der Hepatitis Infectiosa, Deutsche Militärarzt **9**:8, 1944. Neuweiler, W., and Bronz: Ueber die Bedeutung der Leberprüfungsreaktion nach Gros für die Geburtshilfe, Monatsschr. f. Geburtsh. u. Gynäk. **120**:1, 1945. Grinsted, T.: Takata-Ara Reaction as Liver Function Test and Simple Titration as Substitute, Nord. med. **31**:1879, 1946. Bjørnbor, M.: Serum Proteins in Hepatitis: II. Takata Reaction and Gros Reaction, Acta. med. Scandinav. **124**: 466, 1946. Mandel, E. E., and Paris, D. A.: The Routine Use of the Serum Flocculation Reaction with Hayem's Solution, J. Lab. & Clin. Med. **33**:1629, 1948. Mandel, E. E.; Paris, D. A., and Harris, D. T.: Evaluation of the Flocculation Test with Hayem's Solution, ibid. **34**:653, 1949.

48. Bauer, R., and Wozasek, O.: Ueber die $Mg.Cl_2$-Flockungsreaktion im Blutserum nach R. Bauer, Med. Klin. **31**:1076, 1935. Bauer, R.: Eine neue Seroreaktion—Serum-Goldsolreaktion, Klin. Wchnschr. **16**:1570, 1937; Mechanism of Modern Serum Tests in Relation to Their Clinical Significance: Magnesium Chloride, Serum Colloidal Gold, Cephalin-Cholesterol, Turbidity Tests, Rev. Gastroenterol. **16**:158, 1949.

tests all show gross changes in the albumin to globulin ratio. The reactions to the formol-gel and the Neumann dilution tests in my experience usually do not become positive unless there is hyperglobulinemia of 3 per cent or more, which accounts for their use in the diagnosis of such conditions as kala-azar, lymphogranulomatosis and multiple myeloma. These procedures are of less value in the study of hepatic disease. The Takata-Ara reaction and the reactions of Bauer and Weltmann are positive when hyperglobulinemia is present, but this may be relative as well as absolute. In my experience the Takata-Ara test has been the most satisfactory of this group for routine use. A positive reaction usually indicates a reversed albumin to globulin ratio, and this reversal may be due to a reduction in the albumin, as well as to an increase in the globulin fraction. As such, the procedure is not a specific test of hepatic insufficiency. However, when other causes of alterations in the serum proteins are excluded on clinical grounds or when evidence of chronic hepatic disease, i. e., cirrhosis, is present, a positive Takata-Ara reaction is of confirmatory value.

Electrophoretic Studies.—The introduction of electrophoretic methods for the study of the serum proteins has stimulated much investigation. These methods not only provide a sharper separation of albumin from globulin than was possible by the time-honored chemical methods of separation but also permit the study of the various globulin fractions.

A great many investigators have reported changes in the serum proteins in hepatic disease.[50] In acute hepatitis a reduction in albumin

49. Weltmann, O.: Ueber die Spiegelung exsudativ-entzündlicher und fibröser Vorgänge im Blutserum, Med. Klin. **26**:240, 1930. Weltmann, O., and Medvei, C. V.: The Coagulation Band and Its Place in the Clinic, Wien. klin. Wchnschr. **12**:1910, 1933. Levinson, S. A., and Klein, R. I.: The Value of the Weltmann Serum Coagulation Reaction as a Laboratory Diagnostic Aid: Comparison with the Sedimentation Rate, Ann. Int. Med. **12**:1948, 1939. Kraemer, M.: The Weltmann Test in Diseases of the Liver, Am. J. Digest. Dis. **2**:14, 1935; The Serum Coagulation Reaction: Its Clinical Significance, ibid. **9**:129, 1942. Berlin, R.: Weltmann's Serum Coagulation Reaction—A Simple Test of Considerable Clinical Value, Acta med. Scandinav. **122**:281, 1945. Ducci, H., and Yaikin, M.: La suerocoagulación de Weltmann en los enfermedades hepáto-biliares, Rev. méd. de Chile **74**:322, 1946. Siegel, L. J., and Kraemer, M.: Further Studies on the Weltmann Serum Coagulation Reaction, J. Lab. & Clin. Med. **33**:618, 1948.

50. Longsworth, L. G.; Shedlovsky, T., and MacInnes, D. A.: Electrophoretic Patterns of Normal and Pathological Human Blood Serum and Plasma, J. Exper. Med. **70**:399, 1939. Blix, G.: Quantitative Bestimmung von elektrophoretisch getrennten Serumglobulinen, Ztschr. f. d. ges. exper. Med. **105**:595, 1939. Luetscher, J. A., Jr.: Electrophoretic Analysis of Plasma and Urinary Proteins, J. Clin. Investigation **19**:313, 1940. Longsworth, L. G., and MacInnes, D. A.: An Electrophoretic Study of Nephrotic Sera and Urine, J. Exper. Med.

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and an increase in gamma globulin occur. A smaller increase in beta globulin may be present. In portal cirrhosis the reduction in the albumin and the increase in the gamma globulin are more pronounced. Obstructive jaundice produces little change in the protein pattern at the onset, but, as the obstruction continues and hepatic injury results in the development of obstructive biliary cirrhosis, the albumin is reduced and the gamma globulin tends to be elevated. The beta globulin is elevated in cases of biliary cirrhosis. Lipoprotein complexes are an important fraction of the beta globulin component, for extraction with ether removes a considerable proportion of the beta globulin in such cases but does not affect the beta globulin fraction of normal serums.

71:77, 1940. Luetscher, J. A., Jr.: Electrophoretic Analysis of the Proteins of Plasma and Serous Effusions, J. Clin. Investigation **20**:99, 1941. Longsworth, L. G.: Recent Advances in the Study of Proteins by Electrophoresis, Chem. Rev. **30**:323, 1942. Gray, S. J., and Barron, E. S. C.: The Electrophoretic Analysis of the Serum Proteins in Diseases of the Liver, J. Clin. Investigation **22**:191, 1943. Dole, V. P.: The Electrophoretic Patterns of Normal Plasma, ibid. **23**:708, 1944. Zeldis, L. J., and Alling, E. L.: Plasma Protein Metabolism—Electrophoretic Study: Restoration of Circulating Protein Following Acute Depletion by Plasmapheresis, J. Exper. Med. **81**:515, 1945. Thorn, G. W.; Armstrong, S. H., Jr., and Davenport, D. V.: Chemical, Clinical and Immunological Studies of the Products of Human Plasma Fractionation: XXXI. The Use of Salt-Poor Concentrated Human Serum Albumin Solution in the Treatment of Hepatic Cirrhosis, J. Clin. Investigation **25**:304, 1945. Verschure, J. C. M.: Elektrophorese en serumvlokkingsreacties, Amsterdam, Sheltema & Holkema, Ltd., 1946. Malmros, H., and Blix, G.: Plasma Proteins in Cases with High Erythrocyte Sedimentation Rates, Acta med. Scandinav., 1946, supp. 170, p. 280. Martin, N. H.: The Components of the Serum Proteins in Infective Hepatitis and in Homologous Serum Jaundice (an Electrophoretic Study), Brit. J. Exper. Path. **27**:363, 1946. Maclagan, N. F., and Bunn, D.: Flocculation Tests with Electrophoretically Separated Serum Proteins, Biochem. J. **41**:580, 1947. Wunderly, C., and Wuhrmann, F.: The Effect of Experimental Increases in the Gamma-Globulin and Albumin Content of Sera on the Response Given by Turbidity and Flocculation Tests, Brit. J. Exper. Path. **28**:286, 1947. Gutman, A. B.: The Plasma Proteins in Disease, in Anson, M. L., and Edsall, J.: Advances in Protein Chemistry, New York, Academic Press, Inc., vol. 4, p. 155. Cohen, P. P.; Thompson, F. L., and Nitshe, G. A., Jr.: Electrophoretic Analysis of Ante and Post Mortem Serum in Different Diseases, J. Clin. Investigation **26**:821, 1947. Ricketts, W. E.; Sterling, K.; Kirsner, B., and Palmer, W. L.: Electrophoretic Studies of the Serum Proteins in Portal Cirrhosis, Gastroenterology **13**:205, 1949. Sterling, K., and Ricketts, W. E.: Electrophoretic Studies of the Serum Proteins in Biliary Cirrhosis, J. Clin. Investigation **28**:1469, 1949. Ricketts, W. E., and Sterling, K.: Electrophoretic Studies of the Serum Proteins in Virus Hepatitis, ibid. **28**:1477, 1949. Kunkel, H. G., and Ahrens, E. H.: The Relationship Between Serum Lipids and the Electrophoretic Pattern, with Particular Reference to Patients with Primary Biliary Cirrhosis, ibid. **28**:1575, 1949. Sterling, K.: The Serum Proteins in Infectious Mononucleosis: Electrophoretic Studies, ibid. **28**:1057, 1949. Whitman, J. F.: Rossmiller, H. R., and Lewis, L. A.: Protein Alteration in Portal Cirrhosis as Determined by Electrophoresis, J. Lab. & Clin. Med. **35**:167, 1950.

Cephalin Cholesterol Flocculation Test.—The cephalin cholesterol flocculation test was proposed by Hanger in 1938 [51] and has since been widely accepted. The serum of patients with acute parenchymal injury to the liver will flocculate a cephalin cholesterol emulsion. Normal serum or the serum of patients with obstructive jaundice does not flocculate the emulsion.

Hanger and his associates [52] studied the effect on the cephalin cholesterol flocculation of the different fractions of serum obtained by electrophoretic separation. They reported that flocculation is produced by the isolated gamma globulin fraction. The gamma globulin in normal serum does not produce flocculation because of an inhibitory effect of the albumin. A positive reaction in disease may be produced by (1) an increase in the gamma globulin, (2) a decrease in the serum albumin or (3) a change in the flocculation-inhibiting property of the serum albumin. The positive reactions in hepatitis are perhaps due to a combination of all these factors.

It is difficult, therefore, to relate a positive cephalin cholesterol reaction to any specific functional disturbance of the liver except in the most general way. Experience, however, has shown the usefulness of the reaction as an index to parenchymal injury, and its diagnostic value is accepted. Rosenberg [53] reported the test reaction positive in all the patients who had hepatitis with jaundice that he studied. Mateer [35] reported an incidence of positive reactions in 66 to 86 per cent of patients, depending on the type of hepatitis studied. Other investigators have reported reactions positive in 92 to 100 per cent of patients with parenchymal involvement of the liver, depending on the severity of the damage.[54]

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51. Hanger, F. M.: The Flocculation of Cephalin-Cholesterol Emulsions by Pathological Sera, Tr. A. Am. Physicians **53**:148, 1938; Serological Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions, J. Clin. Investigation **18**:261, 1939.

52. Kabat, E. A.; Hanger, F. M.; Moore, D. H., and Landow, H.: The Relation of Cephalin-Flocculation and Colloidal Gold Reactions to the Serum Proteins, J. Clin. Investigation **22**:563, 1943. Moore, D. B.; Pierson, P. S.; Hanger, F. M., and Moore, D. H.: Mechanism of the Positive Cephalin-Cholesterol Flocculation Reaction in Hepatitis, ibid. **24**:292, 1945. Hanger, F. M., and Patek, A. J., Jr.: Cephalin Flocculation Test in Cirrhosis of Liver, Am. J. M. Sc. **202**:48, 1941. Recant, L.; Chargaff, E., and Hanger, F. M.: Comparison of the Cephalin-Cholesterol Flocculation with the Thymol Turbidity Test, Proc. Soc. Exper. Biol. & Med. **60**:245, 1945.

53. Rosenberg, D. H.: Cephalin-Cholesterol Flocculation Test in Cases of Disease of Liver, with Special Reference to Diagnosis of Mild and Unsuspected Forms, Arch. Surg. **43**:231 (Aug.) 1941.

54. Alessandri, H.; Ducci, H., and Galecio, R.: Experiencia clínica con la reacción de Hanger (floculación cefalina-colesterol), Rev. méd. de Chile **70**:848, 1942. Wade, L. J., and Richman, E. E.: Cephalin-Cholesterol Flocculation Test, J. Lab. & Clin. Med. **30**:6, 1945.

Thymol Turbidity Test.—Maclagan in 1944 showed that when a saturated solution of thymol was added to the serum of a patient with hepatitis it became turbid.[55] Studies of the mechanism of this thymol turbidity test showed that an increased concentration of gamma globulin was one factor in producing a positive reaction. An increase of the lipids in the serum, especially those associated with beta globulin in lipoprotein complexes, also favors a positive reaction to the thymol turbidity test.[56]

According to Maclagan, the degree of turbidity present at the end of one-half hour is measured by the formazine turbidity standards of Kingsbury.[57] Values over 4 units are considered positive. Shank and Hoagland [58] used a spectrophotometer to measure the turbidity. Neefe [59] allowed the thymol mixture to stand overnight and measured the degree of flocculation.

Mateer and his associates considered the original test of Maclagan to be relatively insensitive for a screening procedure. They changed the p_H of the buffer from 7.8 to 7.55 and reported that when modified in this manner the thymol turbidity test is quite as sensitive as the cephalin cholesterol flocculation.[35]

A great many studies with the thymol turbidity test have been reported, and its usefulness is now accepted.[60]

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55. Maclagan, N. F.: Thymol Turbidity Test: A New Indicator of Liver Dysfunction, Nature, London **154**:670, 1944; The Thymol Turbidity as an Indicator of Liver Dysfunction, Brit. J. Exper. Path. **25**:234, 1944.

56. Recant, L.; Chargaff, E., and Hanger, F. M.: Comparison of the Cephalin Flocculation with the Thymol Turbidity Test, Proc. Soc. Exper. Biol. & Med. **60**:245, 1945. Watson, C. J., and Rappaport, E. M.: A Comparison of the Results Obtained with the Hanger Cephalin-Cholesterol Flocculation Test and the Maclagan Thymol Turbidity Test in Patients with Liver Disease, J. Lab. & Clin. Med. **30**:893, 1945.

57. Kingsbury, F. B.; Clark, C. P.; Williams, G., and Post, A. L.: The Rapid Determination of Albumin in Urine, J. Lab. & Clin. Med. **11**:981, 1926.

58. Shank, R. E., and Hoagland, C. L.: A Modified Method for the Quantitative Determination of the Thymol Turbidity Reaction of the Serum, J. Biol. Chem. **162**:133, 1946. Kunkel, H. G., and Hoagland, C. L.: Mechanism and Significance of Thymol Turbidity Test for Liver Disease, J. Clin. Investigation **26**:1060, 1947. Ley, A. B.; Lewis, J. H., and Davidson, C. S.: The Quantitative Determination of the Thymol Turbidity Reaction of Serum, J. Lab. & Clin. Med. **31**:910, 1946.

59. Neefe, J. R.: Results of Hepatic Tests in Chronic Hepatitis Without Jaundice, Gastroenterology **7**:1, 1946.

60. (*a*) Havens, W. P., Jr., and March, R. E.: A Comparison of the Cephalin-Cholesterol Flocculation and Thymol Turbidity Tests in Patients with Experimentally Induced Hepatitis, J. Clin. Investigation **25**:816, 1946. (*b*) Cohen, P. P., and Thompson, F. L.: Mechanism of the Thymol Turbidity Test, J. Lab. & Clin. Med. **32**:475, 1947. (*c*) Maclagan, N. F.: Liver Function Tests in Diagnosis of Jaundice: Review of 200 Cases, Brit. M. J. **2**:197, 1947. (*d*) Mann, F. D.; Snell, A. M., and Butt, H. R.: The Thymol Turbidity Test and Impaired Liver Func-

Colloidal Gold Reaction.—The colloidal gold reaction has been applied to blood serum and used in the study of hepatic disease. Maclagan [61] and Grey [62] in particular have emphasized its value.

Studies of the mechanism of the reaction have shown that gamma globulin is the fraction responsible for the coagulation of the gold solution.[63] A lipoprotein fraction associated with beta globulin, on the other hand, has a protective action on the gold solution. If these views on the mechanism of the colloidal gold reaction are correct, it may be assumed that the results obtained with the gold solution will correspond more nearly to the thymol turbidity than to the cephalin cholesterol flocculation.

Various writers have reported that the colloidal gold reaction is a sensitive index to hepatic injury.[64] However, Maclagan [60c] and I are agreed in preferring the cephalin cholesterol flocculation and the thymol turbidity tests for routine use.

tion, Gastroenterology **9**:651, 1947. (*e*) Shay, H.; Berk, J. E., and Siplet, H.: The Thymol Turbidity Test as a Measure of Liver Disease, with Special Reference to Comparison of the Turbidity at 18 Hours to That at 30 Minutes ("18 Hour Turbidity Ratio"), ibid. **9**:641, 1947. (*f*) Neefe, J. R.; Bahnson, E. R., and Reinhold, J. G.: Studies of Responses of Certain Hepatic Tests in Diseases of the Liver and Biliary Tract: I. Serum Cephalin Cholesterol Flocculation, Thymol Turbidity, Thymol Flocculation and Colloidal Gold Responses, ibid. **9**:656, 1947. (*g*) Ducci, H.: The Thymol Test of Maclagan: Standardization and Adaptation to the Evelyn Photoelectric Colorimeter, J. Lab. & Clin. Med. **32**:1267, 1947. (*h*) Linder, H. K.; Bruger, M., and Greene, C. H.: Comparative Studies with Some Newer Tests for Hepatic Dysfunction, Thymol Turbidity, Cephalin-Cholesterol Flocculation and Colloidal Gold Reaction, New York State J. Med. **48**:1371, 1948. (*i*) Kunkel, H. G.: Value and Limitations of Thymol Turbidity Test as Index of Liver Disease, Am. J. Med. **4**:201, 1948. (*j*) Shay, H., and Siplet, H.: Minimal Yet Adequate Program for Liver Function Studies in Differential Diagnosis of Jaundice, ibid. **4**:215, 1948. (*k*) Denninger, K., and Goedtler, A.: Usefulness of Maclagan's Thymol Turbidity Test for Differential Diagnosis of Icterus, Deutsche med. Wchnschr. **74**:326, 1949 [partial index]. (*l*) de la Huerga, J., and Popper, H.: Standardized Reagent for Thymol Turbidity Test, J. Lab. & Clin. Med. **34**:877, 1949.

61. Maclagan, N. F.: The Serum Colloidal Gold Reaction as a Liver Function Test, Brit. J. Exper. Path. **25**:15, 1944.

62. Grey, S. J.: The Colloidal Gold Reaction of Blood Serum in Diseases of the Liver, Arch. Int. Med. **65**:524 (March) 1940; Studies on the Mechanism of the Spinal Fluid Colloidal Gold Reaction, Proc. Soc. Exper. Biol. & Med. **51**:401, 1942.

63. Bernsohn, J., and Borman, E. K.: Proteins in the Colloidal Gold Reaction, J. Clin. Investigation **26**:1026, 1947. Kabat, E. A.; Moore, D. H., and Landow, H.: An Electrophoretic Study of the Protein Components in Cerebrospinal Fluid and Their Relationship to the Serum Proteins, ibid. **21**:571, 1942.

64. Lavín, R.; Sellek Azzi, A., and del Frade, A.: Sobre la reacción del oro coloidal de Lange en el suero sanguíneo para el diagnóstico de las Hepatopatiás (Nota preliminar), Vida nueva **49**:203, 1942. Ducci, H., and Donoso, H.: Experiencia clínica con la reacción del oro coloidal en el suero, especialmente en el estudio de las afecciones hépatobiliares, Stanley, Santiago, Chile, 1946.

Colloidal Red Reaction.—Maizels [65] and Ducci [66] have recently recommended the use of colloidal red precipitation as a flocculation test for hepatic disease. The determination is performed in the same manner as Maclagan's colloidal gold test. Bruger and Oppenheim [67] report that it is a simple and sensitive reaction and in their hands appears to parallel the results of the cephalin cholesterol flocculation and the thymol turbidity tests. Strade and Dotti [68] made similar observations and suggest that the colloidal red and the thymol turbidity tests are primarily measures of antibody globulin, in contrast to those tests which are measures of gamma globulin. The validity of this suggestion still remains to be proved.

Zinc Sulfate Turbidity.—Kunkel studied the precipitation of globulin from the serum by zinc, mercury, lead, cadmium, uranium and copper salts.[69] He observed that when serum was precipitated with a solution containing a small amount of zinc sulfate a turbid suspension was formed. The optical density of this suspension was proportional to the concentration of the gamma globulin present. He reported on a series of normal serums with a range of 4 to 12 units and an average value of 8 units. In acute infectious hepatitis a slight increase was noted in the zinc sulfate turbidity. There was a much greater concentration in cirrhosis. Kunkel has indicated that values for gamma globulin remain elevated late in the convalescent phase of infectious hepatitis and are of especial clinical significance in the evaluation of this period. Cholangitis and the complications of biliary obstruction apparently have less effect on the gamma globulin than they do on the thymol turbidity. Determination of the gamma globulin level, therefore, is claimed to be the more specific test in the differential diagnosis of hepatobiliary disorders.

65. Maizels, M.: Empirical Tests of Liver Function, Lancet **2**:401, 1946.

66. Ducci, H.: The Colloidal Red Test for the Study of Hepatic Dysfunction, J. Lab. & Clin. Med. **32**:1273, 1947; La reacción del rojo coloidal y la adaptación al colorimetro fotoeléctrico de la reacción de enturbiaminento al timol de Maclagan: Communicación preliminar, Rev. méd. de Chile **74**:773, 1946.

67. Bruger, M., and Oppenheim, E.: The Present Status of Liver Function Tests Including Observations on the Newer Flocculation Procedures, Bull. New York Acad. Med. **25**:16, 1949. Oppenheim, E.; Bruger, M., and Frost, E.: The Colloidal Red Test as an Index of Liver Dysfunction, J. Lab. & Clin. Med. **34**:662, 1949.

68. Strade, H. A.; Dotti, L. B., and Ilka, S. J.: A Clinical Evaluation of a New Liver Function Test, the Colloidal Red Test, in Comparison with the Thymol Turbidity Test, Am. J. M. Sc. **217**:448, 1949; A Comparative Study of the Colloidal Red Test in Liver Disease with Special Reference to the Zinc Turbidity, Thymol Turbidity and Bromsulphalein Tests, Gastroenterology **12**:934, 1949.

69. Kunkel, H. G.: Estimation of Alterations of Serum Gamma Globulin by a Turbidimetric Technique, Proc. Soc. Exper. Biol. & Med. **66**:217, 1947. Kunkel, H. G.; Ahrens, E. H., and Eisenmenger, W. J.: Application of Turbidimetric Methods for Estimation of Gamma Globulin and Total Lipid to the Study of Patients with Liver Disease, Gastroenterology **11**:499, 1948.

Comment on Protein Changes.—The results of the electrophoretic studies of the serum proteins, together with the investigations on these various special tests, indicate that there are several changes which take place in association with diseases of the liver. The chief changes apparently are (1) a reduction in the serum albumin, (2) a change in the ability of the albumin to inhibit flocculation of colloids by gamma globulin, (3) an increase in gamma globulin, which has a marked flocculatory effect, and (4) an increase in lipoprotein, which is associated with the beta globulin fraction but may not be identical with it.

These changes affect the reported reactions to the different tests to a varying degree. Unfortunately, too little is known regarding the mechanism of the tests to permit a definitive statement regarding the extent to which each reaction is affected by these variables.

The zinc sulfate turbidity is reported to be the most specific index to changes in the gamma globulins. This perhaps explains in part why it is the least sensitive. Globulins are usually considered to be formed from extrahepatic sources. Luetscher[70] has discussed in some detail the changes in response to infection and associated with immune antibodies. Such effects must be recognized on clinical grounds and excluded before changes are interpreted as specific indications of hepatic disease.

The cephalin cholesterol flocculation test of Hanger shows changes in one or the other of the first three factors listed above.

The mechanism of the colloidal gold and colloidal red tests has been studied in less detail than that of the others. Apparently, they are primarily indexes to changes in the gamma globulin. The thymol turbidity test, on the other hand, is affected by changes in both gamma globulin and the lipoprotein fraction. The exact relation of lipoprotein and beta globulin is still not clear.

To date, it may be accepted that these and other related tests, with the exceptions noted, afford approximate indications of changes in the gamma globulin of the serum.[71] Many yield close approximations, but,

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70. Luetscher, J. A.: Biological and Medical Applications of Electrophoresis, Physiol. Rev. **27**:621, 1947.

71. Cohn, E. J.; Strong, L. E.; Hughes, W. L., Jr.; Mulford, D. J.; Ashworth, J. N.; Melin, M., and Taylor, H. L.: Preparation and Properties of Serum and Plasma Proteins: IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids, J. Am. Chem. Soc. **68**:459, 1946. Wunderly, C., and Wuhrmann, F.: Die Cadmium-Reaktion im Blutserum, Schweiz. med. Wchnschr. **75**:1128, 1945; Effect of Experimental Increases in Globulin and Albumin Content of Sera on Response Given by Turbidity and Flocculation Tests, Brit. J. Exper. Path. **28**:286, 1947. Wuhrmann, F.; Wunderly, C., and Bubb, W.: Zur Prüfung der Kolloid-Labilität des menschlichen Blutserums in der Praxis, Schweiz. med. Wchnschr. **77**:667, 1947. Wuhrmann, F. H., and others: Die Bluteiweisskörper des Menschen: Untersuchungsmethoden und deren klinisch-praktische Bedeutung, Basel, Benno Schwabe, 1947. Wuhrmann, F. H., and Wunderly, C.: The Cadmium Reaction: A Practical Test for

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when tested by electrophoretic methods, the precipitate usually can be shown to contain varying amounts of the other serum proteins. Simple methods for determining the individual protein fractions in the serum with increased specificity, therefore, promise to be of great help in the study of this problem. Further knowledge concerning the origin and metabolic behavior of these different fractions, especially in relation to the formation of antibodies and the various immune reactions, is likewise desirable.[72] Albumin, alpha globulin and specific globulins, such as prothrombin, are produced by the hepatic cells. Gamma globulin is formed by reticuloendothelial cells, by plasma cells and by lymphocytes. The elevation of the gamma globulin in hepatic disease is to be considered the direct result not of damage to parenchymal cells but of stimulation of the Kupffer cells and of other reticuloendothelial and mesenchymal cells by the products of the breakdown of hepatic cells.[73] Such changes, for example, possibly explain the positive reactions obtained in rheumatoid arthritis, rheumatic fever and a variety of other conditions not ordinarily associated with diseases of the liver.[74]

Until these questions are answered in detail, it must be remembered that the clinical use of tests of this type in the diagnosis of hepatic disease is largely empirical. However, this fact does not impair the clinical usefulness of the tests.[75]

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the Evaluation of Serum Lability: Comparison with the Cephalin-Cholesterol Flocculation and Thymol Turbidity Test, J. Lab. & Clin. Med. **34**:1162, 1949. Cohn, C., and Wolfson, W. Q.: Studies in Serum Proteins: I. The Chemical Estimation of Albumin and of the Globulin Fractions in Serum, ibid. **32**:1203, 1947; II. A Rapid Clinical Method for the Accurate Determination of Albumin and Globulin in Serum or Plasma, ibid. **33**:367, 1948.

72. Madden, S. C., and Whipple, G. H.: Plasma Proteins: Their Source, Production and Utilization, Physiol. Rev. **20**:194, 1940. Roberts, S., and White, A.: Studies on the Origin of the Serum Proteins, J. Biol. Chem. **180**:505, 1949. Sabin, F. R.: Cellular Reactions to a Dye Protein with a Concept of the Mechanism of Antibody Formation, J. Exper. Med. **70**:67, 1939. Marrack, J. R., and Hoch, H.: Serum Proteins: A Review, J. Clin. Path. **2**:161, 1949. Metcoff, J., and Stare, F. J.: The Physiologic and Clinical Significance of Plasma Proteins and Protein Metabolites, New England J. Med. **236**:26, 1947.

73. Popper, H.; de la Huerga, J.; Steigmann, F., and Slodki, M.: Turbidimetric Gamma Globulin Determinations in Hepatobiliary Diseases, J. Lab. & Clin. Med. **35**:391, 1950.

74. Nelson, H. G., and Seal, J. R.: Studies on Rheumatic Fever: Comparative Value of the Weltmann Serocoagulation Reaction and the Sedimentation Rate (Cutler) in Determining Activity of the Rheumatic Process, J. Lab. & Clin. Med. **35**:220, 1950.

75. (*a*) Allessandri, H., and Ducci, H.: Diagnóstico diferencial de los cuadros ictéricos: Estudio comparativo de la dosificación de colesterol total, la determinación de fosfatasas, la prueba del benzoato de sodio y la floculación cefalina-colesterol, Rev. méd. de Chile **71**:1150, 1943. (*b*) Watson, C. J., and Rappaport, E. M.: A Comparison of the Results Obtained with the Hanger Cephalin Cholesterol Flocculation Test and the Maclagan Thymol Turbidity Test in Patients

PROTHROMBIN TIME

The demonstration that cholemic bleeding is due to a deficiency of prothrombin, which can be recognized by determining the prothrombin time of the blood and which in the majority of instances can be corrected by the administration of synthetic vitamin K, was one of the great advances in surgery of the liver and biliary tract. This is generally

with Liver Disease, J. Lab. & Clin. Med. **30**:983, 1945. (*c*) Carter, A. B., and Maclagan, N. F.: Some Observations on Liver Function Tests in Diseases Not Primarily Hepatic, Brit. M. J. **2**:80, 1946. (*d*) Neefe, J. R., and Reinhold, J. G.: Laboratory Aids in the Diagnosis and Management of Infectious (Epidemic) Hepatitis, Gastroenterology **7**:393, 1946. (*e*) Havens, W. P., Jr., and Marck, R. E.: A Comparison of the Cephalin-Cholesterol Flocculation and Thymol Turbidity Tests in Patients with Experimentally Induced Infectious Hepatitis, J. Clin. Investigation **25**:816, 1946. (*f*) Watson, C. J., and Hoffbauer, F. W.: Liver Function in Hepatitis, Ann. Int. Med. **26**:813, 1947. (*g*) Wolfson, W. Q.; Cohn, C.; Calvary, E., and Thomas, E. M.: Studies in Serum Proteins: IV. Clinical Studies Employing Rapid Chemical Fractionation Procedures with Particular Reference to the Frequency and Significance of Hypoalbuminemia, J. Lab. & Clin. Med. **33**:1276, 1948. (*h*) Popper, H.; Steigmann, F.; Dyniewicz, H., and Dubin, A.: Use of Thymol Turbidity as Lipid Absorption Test: Experiences with Thymol Turbidity and Zinc Sulfate Turbidity Tests Under Physiologic and Pathologic Conditions, ibid. **34**:105, 1949. (*i*) Ducci, H.: The Contribution of the Laboratory to the Differential Diagnosis of Jaundice, J. A. M. A. **135**:694 (Nov. 15) 1947. (*j*) Kibrick, A. C., and Clements, A. B.: A Comparative Study of the Serum Albumin-Globulin Ratio: The Cephalin-Cholesterol Flocculation and the Thymol Turbidity Tests for Liver Function, J. Lab. & Clin. Med. **33**: 662, 1948. (*k*) Ducci, H.: Sobre el mecanismo de las reacciones de floculación, Rev. méd. de Chile **76**:463, 1948. (*l*) Ducci, H., and Roeschmann, W.: Las reacciones de floculación y enturbiamiento utilizadas en el estudio de las enfermedades hépato-biliares, ibid. **77**:267, 1949. (*m*) Allessandri, H.; Ducci, H., and Roeschmann, W.: Las reacciones de floculación en el diagnóstico differencial de las ictericias, ibid. **77**:319, 1949. (*n*) Hoffbauer, F. W.; Rames, E. D., and Meinert, J. K.: Limitations and Merits of a Single Serum Sample Analysis in the Differential Diagnosis of Jaundice, J. Lab. & Clin. Med. **34**:1259, 1949. (*o*) Kibrick, A. C., and Clements, A. B.: A Comparative Study of the Serum Albumin-Globulin Ratio, the Cephalin-Cholesterol Flocculation and the Thymol Turbidity Tests for Liver Function, ibid. **33**:662, 1948. (*p*) Popper, H.; Steigmann, F.; Dyniewicz, H., and Dubin, A.: The Influence of Ingestion of Various Lipids upon the Thymol Turbidity, ibid. **33**:1630, 1948. (*q*) Steigmann, F.; Popper, H., and Shulman, B. H.: Combination of Flocculation Tests in the Differential Diagnosis of Jaundice, ibid. **33**:1630, 1948. (*r*) Love, E. B., and Mawson, C. A.: Simplified Flocculation Tests for Differential Diagnosis of Jaundice, Lancet **2**:850, 1949. (*s*) Martin, N. H.: The Components of the Serum Proteins in Infective and Homologous Serum Jaundice, Brit. J. Exper. Path. **27**:363, 1946. (*t*) Steigmann, F.; Popper, H.; Hernandez, R., and Shulman, B.: Flocculation Tests in the Diagnosis of Hepato-Biliary Disease, Gastroenterology **13**:9, 1949. (*u*) Maher, F. T.; Snell, A. M., and Mann, F. D.: Turbidimetric Estimation of Serum Colloids in the Differential Diagnosis of Hepatobiliary Disease, ibid. **12**:394, 1949. (*v*) Maher, F. T., and Mann, F. D.: Turbidimetric Estimation of Serum Colloids in Extrahepatic Disease, ibid. **12**:409, 1949.

recognized and need not be documented here.[76] The determination of the prothrombin time of the blood and the prophylactic preoperative administration of vitamin K are well nigh routine measures in most operations on the gallbladder or biliary tract.

The prothrombin deficiency of the great majority of jaundiced patients responds to treatment with vitamin K. If there is sufficient hepatic damage, the liver may be unable to respond to such therapy and the prothrombin time continues to be prolonged. Such a lack of response has generally been accepted as indicative of serious hepatic disease and, therefore, of prognostic significance.[77] Unger and Shapiro give large test doses of vitamin K (75 mg. of synkayvite® [synthetic vitamin K analogue] is administered intravenously every day for five days). After such massive doses of vitamin K, they frequently observe a transitory prolongation of the prothrombin time which they consider significant of disturbed hepatic function.[78] They report that this test is a sensitive indicator of hepatic function and as sensitive as or more sensitive than the sulfobromophthalein and cephalin cholesterol flocculation tests. However, they use diluted plasma (12.5 per cent) to determine the prothrombin time. In my experience this method has been subject to inexplicable variations, and I am as yet unwilling to accept these conclusions of Unger and Shapiro without considerable reservation.

LIPIDS OF THE SERUM

The role of the liver in lipid metabolism has long been recognized, but many of the details have only recently been clarified. This is especially true of studies on the blood lipids and on the changes produced by hepatic disease. Because simple satisfactory micromethods for the determination of cholesterol in the blood were among the first to be developed, the changes in the cholesterol have been studied in greater

76. Greene, C. H.: Liver and Biliary Tract: A Review for 1941, Arch. Int. Med. **69**:691 (April) 1942.

77. Pohle, F. J., and Stewart, J. K.: Observations on Plasma Prothrombin and Effects of Vitamin K in Patients with Liver or Biliary Disease, J. Clin. Investigation **19**:365, 1940. Allen, J. G., and Julian, O. C.: Response of Plasma Prothrombin to Vitamin K Substitute Therapy in Cases of Hepatic Disease, Arch. Surg. **41**:1363 (Dec.) 1940; Prothrombin and Hepatic Function, ibid. **45**:691 (Nov.) 1942. Seligman, A. M.; Hurwitz, A.; Frank, H. A., and Davis, W. A.: The Intravenous Use of Vitamin K, Surg., Gynec. & Obst. **73**:686, 1941.

78. Richards, R. K., and Shapiro, S.: Experimental and Clinical Studies on the Action of High Doses of Hykinone and Other Menadione Derivatives, J. Pharmacol. & Exper. Therap. **84**:93, 1945. Shapiro, S., and Richards, R. K.: The Prothrombin Response to Large Doses of Synthetic Vitamin K in Liver Disease, Ann. Int. Med. **22**:841, 1945. Unger, P. N., and Shapiro, S.: The Prothrombin Response to the Parenteral Administration of Large Doses of Vitamin K in Subjects with Normal Liver Function and in Cases of Liver Disease: A Standard Test for the Estimation of Hepatic Function, J. Clin. Investigation **27**:39, 1948.

detail than have the changes in the other lipid fractions.[79] This literature was summarized by Greene, Holtz and Leahy,[80] who point out that the value for cholesterol in the blood of adults varies between 150 and 230 mg. per 100 cc., whereas the combined cholesterol (esters) value varies between 60 and 120 mg. The ratio of the combined to the total cholesterol in the blood is quite constant, between 40 and 52 per cent. In the serum the ratio is between 65 and 75 per cent.

Patients with evident hepatic damage have a decreased amount of combined cholesterol in the blood. This decrease is sometimes but not always associated with a decrease in the total cholesterol. The ratio, per se, is not of as great diagnostic usefulness as is the total amount of esters present in the blood. Values for cholesterol esters of less than 60 mg. in patients with obstructive jaundice are of some prognostic significance, as the operative mortality was greater in this group of patients than it was in those with normal ester levels.

In uncomplicated obstructive jaundice the combined cholesterol tends to rise in proportion to the rise in total cholesterol, but in hepatic disease the cholesterol esters are prone to disappear from the blood, regardless of the behavior of the total cholesterol.

In a given case a progressive decrease in the values for the cholesterol esters of the blood usually signifies a poor prognosis, whereas a progressive increase indicates improved hepatic function. Wilkinson [81] points out that the rate of fall frequently is more significant than is the exact level present.

79. Feigl, J.: Neue Untersuchungen über akute gelbe Leberatrophie: III. Fette und Lipoide des Blutes, Biochem. Ztschr. **86**:7, 1918. Thannhauser, S. J., and Schaber, H.: Ueber die Beziehungen des Gleichgewichtes Cholesterin und Cholesterinester im Blut und Serum zur Leberfunktion, Klin. Wchnschr. **5**:252, 1926. Adler, A., and Lemmel, H.: Zur feineren Diagnostik der Leberkrankheiten: I. Cholesterin und Cholesterin-ester im Blute, Deutsches Arch. f. klin. Med. **158**: 173, 1928. Gardner, J. H., and Gainsborough, H.: Blood Cholesterol Studies in Biliary and Hepatic Disease, Quart. J. Med. **23**:465, 1930. Epstein, E. Z.: Cholesterol Partition in Blood Plasma in Parenchymatous Diseases of the Liver, Arch. Int. Med. **47**:82 (Jan.) 1931. Heinlein, H.: Die Rolle des Leber im Cholesterin- und Phosphatidstoffwechsel, Ztschr. f. d. ges. exper. Med. **91**:638, 1933. Hawkins, W. B., and Wright, A.: Blood Plasma Cholesterol: Fluctuations Due to Liver Injury and Bile Duct Obstruction, J. Exper. Med. **59**:427, 1934. Epstein, E. Z., and Greenspan, E. B.: Clinical Significance of the Cholesterol Partition of the Blood Plasma in Hepatic and in Biliary Diseases, Arch. Int. Med. **58**:860 (Nov.) 1936. Jones, C. A.: A Clinical and Laboratory Study of Plasma Lipids in Obstructive Jaundice and Several Types of Hepatic Disease, Am. J. Digest. Dis. **9**:1, 1942. Peters, J. P., and Man, E. B.: The Interrelations of Serum Lipids in Normal Persons, J. Clin. Investigation **22**:707, 1943.

80. Greene, C. H.; Holtz, R., and Leahy, E.: Clinical Value of Determination of Cholesterol Esters of Blood in Hepatic Disease, Arch. Int. Med. **65**:524 (March) 1940.

81. Wilkinson, C. F., Jr.: Personal communication to the author.

Obstruction of the biliary passages leads regularly to an increase in the cholesterol, the phospholipids and, in smaller measure, the neutral fat of the serum. Cholesterol esters may be increased but to a lesser extent than the total cholesterol, so that the ratio is reduced. The reduction in cholesterol esters to less than 60 mg. in obstructive jaundice is of some prognostic significance, in that the surgical mortality was greater in such patients than it was in those with normal esters.

In biliary cirrhosis the changes are even more striking because of the presence of pronounced hyperlipemia.[82] The increase is greater in the phospholipids and the free cholesterol, with a lesser increase in the neutral fat. Ahrens and Kunkel [83] stress the association of cutaneous xanthomas with total lipid levels above 2,000 mg. per 100 cc. The serum in these patients is clear and not lipemic in appearance. Ahrens and Kunkel point out the findings in the serum as characteristic and contrast them with changes seen in other types of hyperlipemia, such as nephrosis, von Gierke's disease (glycogenosis) and essential xanthomatosis, in which the hyperlipemia is due primarily to an increase in

82. Chanutin, A., and Ludewig, S.: Blood Lipid Studies in a Case of Xanthomatosis Associated with Hepatic Damage, J. Lab. & Clin. Med. **22**:903, 1936. Weidman, F. D., and Boston, L. N.: Generalized Xanthoma Tuberosum with Xanthomatous Changes in Fresh Scars of an Intercurrent Zoster: Adenocarcinoma of Ampulla of Vater at Necropsy, Arch. Int. Med. **59**:793 (May) 1937. Thannhauser, S. J., and Magendantz, H.: The Different Clinical Groups of Xanthomatous Diseases: A Clinical Physiological Study of 22 Cases, Ann. Int. Med. **11**: 1662, 1938. Jones, C. D.: Clinical and Laboratory Study of Plasma Lipids in Obstructive Jaundice and Several Types of Liver Disease, Am. J. Digest. Dis. **9**:1, 1942. Eusterman, G. B., and Montgomery, H.: Disorders of the Liver and Extrahepatic Biliary Ducts Associated with Cutaneous Xanthomas and Hyperlipemia, Gastroenterology **3**:275, 1944. Man, E. B.; Kartin, B. L.; Durlacher, S. H., and Peters, J. P.: The Lipids of Serum and Liver in Patients with Hepatic Diseases, J. Clin. Investigation **24**:623, 1945. Thannhauser, S. J.: Medical Progress: Serum Lipids and Their Value in Diagnosis, New England J. Med. **237**: 515 and 546, 1947. Varela Fuentes, B.; Velasco Suarez, C., and Del Campo, J. C.: Cirrosis biliar xanthomatosa, in Varela Fuentes, B., and Munilla, A.: Pathología Digestiva, Buenos Aires, Espasa-Calpe, S. A., 1947, vol. 2, p. 507. MacMahon, H. E., and Thannhauser, S. J.: Xanthomatous Biliary Cirrhosis (a Clinical Syndrome), Ann. Int. Med. **30**:121, 1949.

83. Kunkel, H. G.: Estimation of Alterations of Serum Gamma Globulin by a Turbidimetric Technique, Proc. Soc. Exper. Biol. & Med. **66**:217, 1947. Aherns, E. H., Jr., and Kunkel, H. G.: The Stabilization of Serum Lipid Emulsions by Serum Phospholipids, J. Exper. Med. **90**:409, 1949; The Relationship Between Serum Lipids and Skin Xanthomata in 18 Patients with Primary Biliary Cirrhosis, J. Clin. Investigation **28**:1565 (Nov.) 1949. Kunkel, H. G., and Ahrens, E. H.: The Relationship Between Serum Lipids and the Electrophoretic Pattern, with Particular Reference to Patients with Primary Biliary Cirrhosis, ibid. **28**:1575, 1949. Ahrens, E. H., Jr.: The Lipid Disturbance in Biliary Obstruction and Its Relationship to the Genesis of Arteriosclerosis, Bull. New York Acad. Med. **26**:161, 1950.

neutral fat and the serum appears lipemic. Other investigators unfortunately have not observed the changes in the lipid distribution to be as specific as indicated by Ahrens and Kunkel.

In acute infectious hepatitis the cholesterol tends to be normal or slightly reduced, with greater reduction in the cholesterol esters. The phosphatids, on the other hand, are slightly increased, as is the neutral fat.

In portal cirrhosis the tendency seems to be for all components to fall within or below the limits of normal, though the lipids may be increased slightly in occasional cases. In general, the values for the total lipids decrease *pari passu* with the decrease in the serum albumin.

Kunkel and Ahrens have emphasized the clinical value of measuring the serum lipids in cases of hepatic disease. They describe a turbidimetric method for determining the total lipids, using a reagent of 1 per cent phenol and 12 per cent sodium chloride in distilled water.

Kunkel, Ahrens and Eisenmenger [84] consider that changes in the total lipids as shown by this method are somewhat more specific for disease of the liver than are changes in the gamma globulin. However, Maher, Snell and Mann [85] and Maher and Mann [75v] report considerably greater variation in the total lipids. They also report that in their hands the results of the turbidimetric method for determining total lipids were 10 to 15 per cent higher than those obtained by chemical analysis.

HIPPURIC ACID SYNTHESIS

The synthesis of hippuric acid was introduced by Quick [86] as a test of hepatic function. As such it has been generally accepted. The intravenous test, according to Mateer,[35] yields a higher percentage of positive results than the oral test. However, comparative studies [87] indicate that the hippuric acid test is less sensitive than the other tests discussed here and the technic is not as simple as that of the various flocculation tests. I personally prefer the latter for routine use.

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84. Kunkel, H. G.; Ahrens, E. H., and Eisenmenger, W. J.: Application of Turbidimetric Methods for Estimation of Gamma Globulin and Total Lipid to the Study of Patients with Liver Disease, Gastroenterology **11**:499, 1948.

85. Maher, F. T.; Snell, A. M., and Mann, F. D.: Turbidimetric Estimation of Serum Colloids in the Differential Diagnosis of Hepatobiliary Disease, Gastroenterology **12**:394, 1949.

86. Quick, A. J.: The Synthesis of Hippuric Acid: A New Test of Liver Function, Am. J. M. Sc. **185**:630, 1933; Intravenous Modification of the Hippuric Acid Test of Liver Function, Am. J. Digest. Dis. **6**:716, 1939.

87. Snell, A. M., and Plunkett, J. E.: A Hippuric Acid Test for Hepatic Function: Its Relation to Other Tests in General Use, Am. J. Digest. Dis. **2**:716, 1936. Neefe, J. R.; Stokes, J., Jr.; Reinhold, J. G., and Lukens, F. D. W.: Hepatitis Due to the Injection of Homologous Blood Products in Human Volunteers, J. Clin. Investigation **23**:836, 1944.

ALKALINE PHOSPHATASE OF THE SERUM

The clinical usefulness of determining the alkaline phosphatase of the serum as an aid to the differential diagnosis of jaundice has been a source of controversy since it was first advocated by Roberts.[88] It is recognized that the phosphatase content of the serum is nearly always elevated in cases of complete biliary obstruction [89] such as that due to carcinoma. Gutman and his associates [90] recorded elevated values in over 90 per cent of patients with calculous obstruction of the common duct. Gutman pointed out that the frequency distribution of values for alkaline phosphatase in hepatitis shows a double peak. In the majority of cases of hepatogenous jaundice values for alkaline phosphatase lie within the normal range. In a second group of cases values may be quite as elevated as those seen in cases of obstructive jaundice. It was the presence of this group of cases then unrecognized that led me originally to

88. Roberts, W. M.: Blood Phosphatase and van den Bergh Reaction in Differentiation of Several Types of Jaundice, Brit. M. J. **1**:734, 1933.

89. Bodansky, A., and Jaffe, H. L.: Phosphatase Studies: IV. Serum Phosphatase of Non-Osseous Origin; Significance of Variations of Serum Phosphatase in Jaundice, Proc. Soc. Exper. Biol. & Med. **31**:107, 1933. Herbert, F. K.: Plasma Phosphatase in Various Types of Jaundice, Brit. J. Exper. Path. **16**:364, 1935. Anderson, R. G.: Plasma Phosphatase in Jaundice, St. Barth. Hosp. Rep. **68**:221, 1935. Rothman, M. M.; Meranze, D. R., and Meranze, T.: Blood Phosphatase as Aid in Differential Diagnosis of Jaundice, Am. J. M. Sc. **192**:526, 1936. Cantarow, A., and Nelson, J.: Serum Phosphatase in Jaundice, Arch. Int. Med. **59**:1045 (June) 1937. Bodansky, A.: Non-Osseous Origins of Serum Phosphatase: The Liver, Enzymologia **3**:258, 1937. Shay, H., and and Fieman, P.: Value of Combined Study of Newer Laboratory Tests in Differential Diagnosis of Toxic and Obstructive Jaundice Including Blood Phosphatase, Cholesterol Partition, Galactose Tolerance and Glucose Tolerance, Am. J. Digest. Dis. **5**:597, 1938. Meranze, D. R.; Meranze, T., and Rothman, M. M.: Serum Phosphatase as Aid in Diagnosis of Metastasis of Cancer to Liver, Pennsylvania M. J. **41**:1160, 1938. Cantarow, A.: Biliary Stasis and Decompression: Review of Recent Contributions, Internat. Clin. **1**:272, 1938. Meranze, T.; Meranze, D. R., and Rothman, M. M.: Review of the Value of Serum Phosphatase in Diseases of the Liver, Rev. Gastroenterol. **6**:254, 1939. Giordano, A. S.; Wilhelm, A., and Prestud, M. C.: The Serum Phosphatase in the Differential Diagnosis of Obstructive Jaundice, Am. J. Clin. Path. **9**:226, 1939. Winkelman, L., and Schiffmann, A.: Clinical Significance of Variations in Serum Phosphatase in Hepatic and Biliary Disorders, Arch. Int. Med. **64**:348 (Aug.) 1939. Cantarow, A., and Trumper, M.: Clinical Biochemistry, ed. 2, Philadelphia, W. B. Saunders Company, 1939. García Romeu, D.; del Portillo, L., and Piedra, J. H.: Contribución estudio de las fosfatasas séricas: Informe preliminar, Vida nueva **49**:121, 1942.

90. Flood, C. A.; Gutman, E. B., and Gutman, A. B.: Phosphatase Activity, Inorganic Phosphorus and Calcium of Serum in Disease of Liver and Biliary Tract; Study of 123 Cases, Arch. Int. Med. **59**:981 (June) 1937. Gutman, A. B.; Olson, K. B.; Gutman, E. B., and Flood, C. A.: Effect of Disease of the Liver and Biliary Tract upon the Phosphatase Activity of the Serum, J. Clin. Investigation **19**:129, 1940.

question the diagnostic value of this test.[91] Further experience with the interpretation of this test in the light of the reports of Hanger and Gutman [91a] has convinced me that the difficulty was not with the test but with the interpretation put on it at that time.

The studies of Hanger and Gutman [92] and of Watson and Hoffbauer,[93] in particular, have emphasized the importance of cholangiolitis in producing obstruction of the finer radicles of the bile ducts in the liver. It is in patients with such intrahepatic obstruction that the laboratory tests show an elevation of the alkaline phosphatase of the serum in association with acute or subacute hepatitis. Once the existence of this condition is admitted, it is soon recognized that characteristic symptoms, particularly pruritus, are present to aid in the clinical diagnosis of the intrahepatic obstruction.

The localization of alkaline phosphatase in the liver may be studied by the histochemical method of Gomori.[94] In the normal liver phosphatase activity is limited to the Kupffer cells, to the reticulum lining the sinuses and to the bile canaliculi. The cytoplasm of the liver cells is devoid of activity, and only traces are present in the nuclei of the hepatic cells.[95] In obstructive jaundice, the bile canaliculi are dilated

91. Greene, C. H.; Shattuck, H. F., and Kaplowitz, L.: Phosphatase Content of Blood Serum in Jaundice, J. Clin. Investigation **13**:1079, 1934.

91a. Footnote 90. Hanger, F. M. Jr., and Gutman, A. B.: J. A. M. A. **115**:263 (July 27) 1940.

92. Hanger, F. M., Jr., and Gutman, A. B.: Postarsphenamine Jaundice Apparently Due to Obstruction of Intrahepatic Biliary Tract, J. A. M. A. **115**: 263 (July 27) 1940.

93. Watson, C. J., and Hoffbauer, F. W.: The Problem of Prolonged Hepatitis with Particular Reference to the Cholangiolitic Type and to the Development of Cholangiolitic Cirrhosis of the Liver, Ann. Int. Med. **25**:195, 1946.

94. Gomori, G.: Microtechnical Demonstration of Phosphatase in the Tissue Sections, Proc. Soc. Exper. Biol. & Med. **42**:23, 1939; The Distribution of Phosphatase in Normal Organs and Tissues, J. Cell. & Comp. Physiol. **17**:71, 1941. Takamatsu, H., and Otsuki, T.: Untersuchungen über die Phosphatasereaktion der Leberzellen der normalen und der mit o-Amidoazotuol-Diät gefütterten Ratten, Gann **35**:283, 1941.

95. Kabat, E. A., and Furth, J.: A Histochemical Study of the Distribution of Alkaline Phosphatase in Various Normal and Neoplastic Tissues, Am. J. Path. **17**:303, 1941. Bourne, G.: The Distribution of Alkaline Phosphatase in Various Tissues, Quart. J. Exper. Physiol. **32**:1, 1943. Deane, H. W., and Dempsey, E. W.: The Localization of Phosphatases in the Golgi Region of Intestinal and Other Epithelial Cells, Anat. Rec. **93**:401, 1945. Wachstein, M.: Influence of Dietary Deficiencies and Various Poisons on the Histochemical Distribution of Phosphatase in the Liver, Arch. Path. **40**:57 (July) 1945. Wachstein, M., and Zak, F. G.: Histochemical Distribution of Alkaline Phosphatase in Dog Liver After Experimental Biliary Obstruction, Proc. Soc. Exper. Biol. & Med. **62**:73, 1946; Distribution of Alkaline Phosphatase in the Human Liver: Study of Postmortem Material, Arch. Path. **42**:50 (Nov.) 1946. Jacoby, F.: Use of the Phosphatase Reaction in a Method of Demonstrating Bile Capillaries in Rats, J. Physiol. **106**: 23P, 1947. Kritzler, R. A., and Beaubein, J.: Microchemical Variation of Alkaline Phosphatase Activity of Liver in Obstructive and Hepatocellular Jaundice, Am. J. Path. **25**:1079, 1949.

and the total phosphatase activity present is increased. The liver cells are still devoid of activity. These changes are most notable in the human liver and the liver of the dog. In cats,[96] the alkaline phosphatase of the serum is only slightly increased after biliary obstruction and the liver shows relatively little change in phosphatase. In these animals the renal glomerulus shows conspicuous enzyme activity, and it would seem that the renal excretion of excess alkaline phosphatase in cats may explain the differences observed between the cat, on one hand, and man and the dog, on the other.

Wackstein and Zak[97] report that these histochemical methods permitted them to demonstrate the presence of intracellular bile canaliculi in the rabbit liver. The illustrations they publish look remarkably like those which Grafflin[37] reported as artefacts. Further study is needed before the presence of such intracellular bile canaliculi can be accepted as unquestionable.

Alkaline phosphatase is demonstrable in the cytoplasm of necrotic or damaged hepatic cells, in experimental chloroform and carbon tetrachloride poisoning in dogs and in hepatic or subacute yellow atrophy in man. The changes in the cirrhotic liver are less remarkable. There are several explanations for such observations, and to date there is no agreement among the various observers.

With the use of the alkaline phosphatase of the serum as a measure of biliary obstruction, the elevated values sometimes observed in portal cirrhosis, or in hepatic metastases, are to be explained as due to intrahepatic obstruction. Patients with external biliary fistulas frequently show elevation of the phosphatase. This observation has been cited as evidence of the unreliability of the test. It seems to me that the test is a measure of the cholangitis and associated obstruction of the finer bile radicles that accompanies the biliary fistula.

COMMENT

The list of possible functions of the liver is a long one; that of the tests which have been proposed to permit the study of one or more of these various activities is even longer. The reaction of the medical student or of the practitioner confronted with such a list too frequently is one of confusion and bewilderment. No set group of tests or schema

96. Cantarow, A.; Stewart, H. L., and McCool, S. G.: Serum Phosphatase in Cats with Total Bile Stasis, Proc. Soc. Exper. Biol. & Med. **35**:87, 1936-1937. Flood, C. A.; Gutman, E. B., and Gutman, A. B.: Serum and Urine Phosphatase Activity in the Cat After Ligation of the Common Bile Duct, Am. J. Physiol. **120**:696, 1937. Cantarow, A.: The Influence of Icteric Sera with High Phosphatase Activity on the Activity of Normal Sera, Am. J. Clin. Path. **10**:858, 1940.

97. Wackstein, M., and Zak, F. G.: Intracellular Bile Canaliculi in the Rabbit Liver, Proc. Soc. Exper. Biol. & Med. **72**:234, 1949.

of examination can be applied in all cases. The selection of tests to be used in any individual patient, rather, is determined by the particular diagnostic problem for which the clinician seeks a solution.

Particularly to be kept in mind is the dissociation of the functions of the liver. It has been emphasized repeatedly that different functions are affected to a different degree by various pathological conditions, and changes in one test reaction are not necessarily paralleled by changes in the reaction to a test indicative of a different type of functional disturbance. Parallel studies with various tests of this type, as those of Neefe on acute virus hepatitis, are of great value in showing the order in which various functions of the liver are involved and the order in which the latter commonly return to normal after injury.

Comparative studies of this type are of limited value in indicating the relative sensitivity or importance of different tests, since no one procedure will measure the functions of the liver as a whole. Under these conditions a variety of tests indicative of the different types of functional disturbances is desirable in the study of any individual patient.

On the other hand, as Lichtman has emphasized, one must consider the association of functional tests. Several tests may measure the same or closely related changes in the organism. The flocculation tests, which are dependent on changes in the serum proteins, or the excretory tests of hepatic function with various dyes are examples of this association. When one is dealing with a group of tests indicative of a single type of functional disturbance, comparative tests are of great value in selecting the best test for routine use. The sensitivity, the specificity and the simplicity of the laboratory procedure must be considered before the final test is selected. Thus, among the various excretory tests of liver function the sulfobromophthalein test has generally been found to be preferable to the bilirubin excretion curve, because not only is bilirubin expensive but the solution for intravenous use is troublesome to prepare. Here, too, a venipuncture is easier for the patient than a duodenal intubation, so that the degree of sulfobromophthalein retention of the blood is usually preferred to the study of its excretion time in the bile. Likewise, at the onset of jaundice, the appearance of bilirubin in the urine or of a direct van den Bergh reaction in the blood serum usually precedes a significant elevation of the icterus index or of the serum bilirubin.

The technic of a test enters into the final choice. Particularly when large numbers of patients are under consideration, a test that can be done with the maximum ease and a minimal degree of cooperation from the patient frequently is preferable to another test which may give more accurate results but in which expensive and delicate apparatus or elaborate laboratory procedures are required. Frequently, the first test can be used as a screen to select those patients in whom study by the second method is desirable.

The studies of Neefe were concerned with the investigation of experimental virus hepatitis. He showed that the appearance of bilirubin in the urine and of a direct van den Bergh reaction in the blood serum were the first laboratory signs of incipient jaundice. These were followed in short order by elevation of the icterus index and serum bilirubin, by a positive cephalin cholesterol flocculation and by retention of sulfobromophthalein in the blood serum.

As the hepatitis subsided and the jaundice cleared, the icterus index and the serum bilirubin returned to normal before the van den Bergh reaction became indirect. The sulfobromophthalein retention and the cephalin cholesterol flocculation became normal about the time bilirubinuria ceased. The thymol turbidity, however, frequently was the last to return to normal.

In cases of suspected industrial poisoning, as in certain types of munitions plants, large numbers of workers were exposed to hepatotoxic chemicals in undesirable amounts. The problem of the plant physicians in those circumstances was that of recognizing cases of possible hepatic injury at the earliest moment. They found that urine specimens could be obtained from the laborers with the least amount of effort. The presence of bilirubin or of urobilinogen in increased amounts then served to indicate those cases in which a careful physical examination and more detailed laboratory study were needed.

Not infrequently a patient is seen in whom examination shows no jaundice, but the liver is enlarged or irregular. The final diagnosis then may rest between cirrhosis and carcinoma of indeterminate origin. Here functional tests are of value, for cirrhosis is a disease characterized by disturbance of the entire liver. Therefore, the results of the sulfobromophthalein test, the cephalin or other flocculation tests and the urinary urobilinogen are all likely to be positive. A metastatic nodule or two may not interfere with the rest of the liver, and the functional reserve of the organ is so great as to preclude changes in even the most sensitive tests. Normal results of functional tests, therefore, speak for a diagnosis of metastatic cancer, when this particular problem in differential diagnosis is under consideration. On the other hand, it has been shown that in cases of primary cancer of the bowel the finding of sulfobromophthalein retention usually indicates the presence of extensive metastasis to the liver.

THE HEPATIC STAR IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

The most frequent use of hepatic function tests is in the differential diagnosis of jaundice. My experience has shown that reliance should be placed not on any single test but on the study of the patient by a variety of tests, the results of which are indicative of different types of

change. In correlating such results, I have found it helpful to use a somewhat arbitrary schema.

The differential diagnosis of jaundice is based on, first, the jaundice; second, the evidences of acute injury to the parenchymal cells of the liver; third, the evidences of chronic injury to the liver with scarring; fourth, the evidences of obstruction of the common or extrahepatic bile ducts, and, fifth, the evidences of obstruction to the intrahepatic bile ducts, i. e., cholangiolitic obstruction. These terms are perhaps to be considered more in a clinical sense than as precise pathological entities. However, they are sufficiently characteristic to serve for a diagnostic schema. For

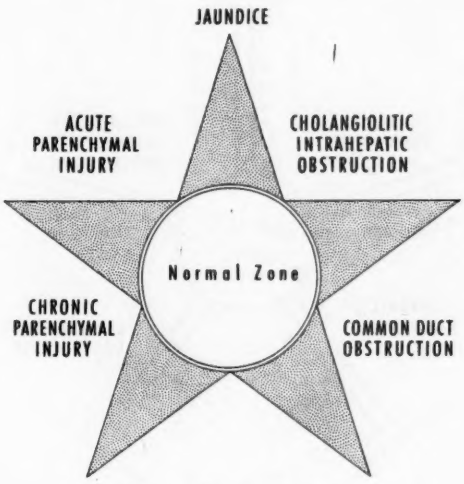

Fig. 1.—The hepatic star.

purposes of illustration they may be remembered as corresponding to the points of a star (fig. 1).

Certain clinical signs may be taken as characteristic accompaniments of each of the processes. The discoloration of the skin and urine in jaundice needs no special comment. In cases of acute hepatitis the associated gastroenteritis and the presence of vascular spiders or of red "liver palms" are generally accepted signs. In chronic injury to the liver or cirrhosis, the presence of ascites, of dilated veins or of splenomegaly is usually evidence of a scarred liver with changes in the portal circulation.

When the stools are clay colored or acholic, it is usually agreed that there is obstruction to the common duct. If the acholia of the stools

is continuous, malignant obstruction is suspected, whereas intermittent obstruction is more suggestive of the presence of a calculus. Pruritus, with its attendant scratching, is a common symptom in obstruction of the common bile duct. If it can be shown that the common bile duct is patent, this symptom points toward an intrahepatic or cholangiolitic obstruction (fig. 2).

An effort has been made in routine work to choose functional tests which are more or less indicative of each of these various processes. Much weight has been given to the ease and simplicity of technic so as to select tests available for bedside use, or at least requiring the

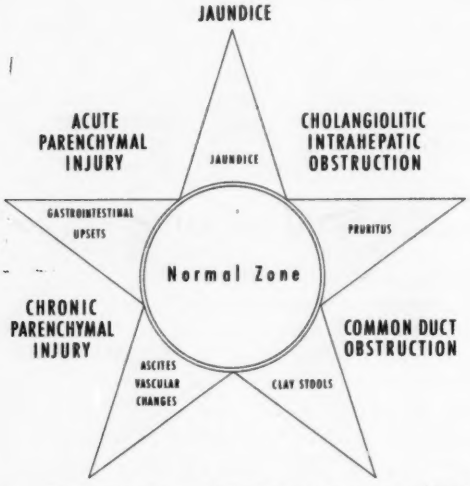

Fig. 2.—The hepatic star in obstruction of the common bile duct.

minimum of laboratory skill. In each category two different tests have been designated which give similar but no necessarily identical indications. Any user may make changes or substitutions in this selection of functional tests without affecting the validity of the schema as a whole (fig. 3).

The intensity of jaundice can be measured by the icterus index or by the serum bilirubin. The 60 second bilirubin test or the determination of the intensity of the biliuria are alternate procedures. I have preferred the cephalin cholesterol flocculation reaction of Hanger and the thymol turbidity reaction of Maclagan as indicative of acute parenchymal injury. A great variety of alternative tests of this type have been proposed and were discussed previously.

The changes in the serum proteins, and particularly the reduction in the serum albumin, are commonly accepted as a crucial sign in chronic hepatitis or cirrhosis. The electrophoretic study of the serum proteins is generally accepted as the most accurate at present, but the expense of the apparatus and the difficulty of study preclude the use of this method in the average hospital. Chemical separation and determination of the serum albumin are less accurate than the electrophoretic study but are available to many more clinicians. Flocculation tests, such as the Takata-Ara reaction, are only qualitative and lack specificity as tests of

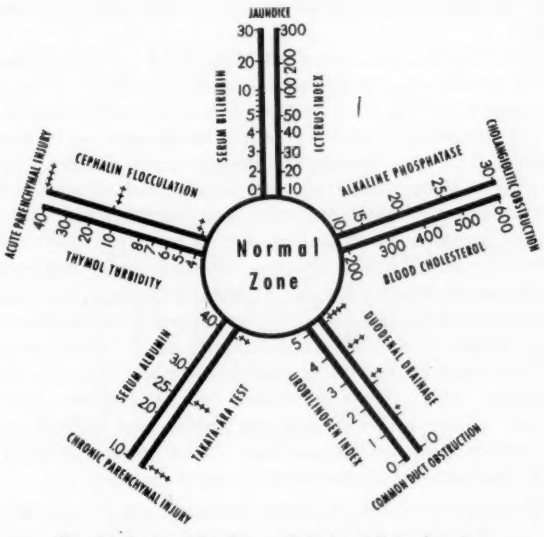

Fig. 3.—Suggested schema of tests of liver function.

hepatic function. However, the technic is simple, and they do show pronounced changes in the albumin to globulin ratio, so that in cases of suspected cirrhosis they are of value in selecting cases for study by more precise methods.

Urobilin or urobilinogen in the urine in a case of jaundice is most commonly evidence of the passage of bile into the intestine. When urobilin is shown to be absent by repeated qualitative tests on different days, or the Watson quantitative test discloses less than 3 units in 24 hours, complete obstruction of the common duct is presumed. It is my opinion, however, that duodenal drainage gives more information regarding the flow of bile through the common duct than any other

single procedure. If a free flow of bile of normal concentration is obtained, the patency of the common duct is established. The presence of Escherichia coli or of Eberthella typhosa in cultures usually indicates infection of the biliary passages. Crystals of calcium bilirubinate, especially if associated with colon bacilli are pathognomonic of stone. Total absence of bile from the duodenal content on repeated examinations, and when the position of the tube has been checked fluoroscopically, establishes the completeness of the obstruction. Such a complete obstruction usually is carcinomatous. Traces of blood in the duodenal content are suggestive of malignant growths. The demonstration of cancer cells by the Papanicolaou method is conclusive evidence of a malignant condition.

Elevations in the total cholesterol of the blood and the alkaline phosphatase of the serum are evidences of obstructive jaundice. If the patency of the common duct has been established by study of the duodenal drainage, such elevation is evidence of an intrahepatic or cholangiolitic obstruction of the type described by Watson and Hoffbauer. Elevation in the alkaline phosphatase level, with the exception of cases of Paget's disease (osteitis deformans) and other bone conditions which are readily recognized clinically, is perhaps more specific for biliary obstruction than are elevations in the blood cholesterol. Both are useful diagnostic tests.

When classified in this fashion, it is evident that there is a correspondence between the significance of the clinical signs in the jaundiced patient and the results of the different laboratory tests. Furthermore, this method of study emphasizes certain characteristic changes which are of diagnostic value. These are shown visually in figures 4 to 12, in which the various points of each star indicate the different types of process which have been discussed and the abnormal findings are indicated by shading of the corresponding point of the star.

1. In an uncomplicated hemolytic jaundice (case 1), only the icterus index and the serum bilirubin are elevated and the van den Bergh reaction is indirect. The other tests listed give normal reactions, for there is no disturbance of either the parenchymal cells of the liver or the biliary radicles (fig. 4).

CASE 1.—*Congenital Hemolytic Jaundice.*—A 16 year old Italian school girl was brought to the hospital in 1946 because of recurring episodes of chills, malaise and dark urine. These attacks were first noted at the age of 14 years and apparently increased progressively in both intensity and frequency.

She had slight icterus. Between attacks the liver and spleen were not palpable, but, after an attack, the liver extended 1 cm., and the spleen 2 cm., below the costal margin. She had a systolic murmur at the apex. She showed no spider angiomas and had no pruritus.

The icterus index varied from 15 to 24 units and was highest just after an attack. Repeated examinations showed that the darkness of the urine was not

due to blood. Following attacks, the urinary urobilinogen secretion was increased to 50.4 mg. per 24 hours. Bile pigment was absent. The van den Bergh reaction of the serum was consistently indirect. The erythrocyte fragility was increased on one occasion, with hemolysis taking place in 0.585 to 0.315 per cent sodium chloride solutions, as compared with a normal value of 0.495 to 0.315 per cent solutions. Spherocytes were observed in the blood smear in small numbers.

The attacks continued to occur at intervals of two to four months, and a splenectomy was done in July 1947. The pathological report confirmed the clinical diagnosis. Since the operation, her condition has been improved. The hemolytic crises still occur, but less frequently. The laboratory findings are unchanged.

Comments.—The above represents a typical case of congenital hemolytic jaundice without a familial history. Splenectomy improves but does not cure the

Fig. 4.—The hepatic star in uncomplicated hemolytic jaundice.

basic defect in the structure of the erythrocytes. The laboratory findings are those of excessive production of bilirubin without disturbance in the liver.

2. In acute hepatitis (case 2) two points of the star are affected, for the jaundice is accompanied with acute parenchymal injury. This star does not distinguish among the various agents producing the acute injury. Similar indications will be obtained in virus diseases, such as acute infectious hepatitis or homologous serum jaundice, and in chemical injury, as in acute poisoning from carbon tetrachloride, or other industrial poisoning. The severity of the damage must also be judged by other criteria than those listed here (fig. 5).

CASE 2.—*Acute Hepatitis (Viral).*—A 48 year old white man was admitted to the University Hospital on May 25, 1949 because of pain in the right upper abdominal quadrant of four weeks' duration. The pain radiated to the back and

was worse on sitting. Two weeks prior to his admission he noted progressive icterus, light yellow stools and dark brown urine. There was no previous history of jaundice, transfusions or plasma injections. He lost 16 pounds (7.3 Kg.) in four weeks.

The results of physical examination were essentially normal, except for the icterus. Duodenal drainage revealed bile, but no concentrated bile was obtained. A culture yielded no pathogenic organisms.

Laboratory tests showed the following values:

	Admission	3 Wk. Later
Icterus index, units	160	188
Total bilirubin, mg./100 cc.	18.0	24.8
Total cholesterol, mg./100 cc.	160	...
Cholesterol esters, mg./100 cc.	40	...
Esters to total cholesterol ratio, %	25	...
Cephalin cholesterol flocculation	4+	4+
Thymol turbidity, units	7.5	10.5
Thymol flocculation	3+	1+
Serum alkaline phosphatase, Bodansky units	7.7	9.4

A medical regimen directed toward treatment of his hepatitis did not elicit a response. The icterus index and alkaline phosphatase level showed some increase three weeks after admission. There was a variance of opinion whether surgical exploration should be carried out. Arguments in favor of surgical exploration were the lack of response to medical therapy, with increasing jaundice and serum alkaline phosphatase. Arguments against surgical intervention were the presence of obvious parenchymal cell damage, the free flow of bile as shown on duodenal drainage and the alkaline phosphatase of less than 10 units. Exploratory laparotomy was eventually carried out.

At operation the liver showed evidence of acute hepatitis; no abnormalities were noted in the gallbladder or common duct. A T tube was inserted.

The patient did well following operation and was discharged from the hospital. Follow-up studies in the clinic after operation revealed the cholesterol to measure 304 mg. and the esters 181 mg. per 100 cc. The icterus index was 24 units and thymol turbidity 10 units, with a trace of thymol flocculation and 1+ cephalin flocculation. The alkaline phosphatase level was 4.0 Bodansky units.

Comment.—This case is an example of a small group of cases of acute hepatitis in which a high icterus index is maintained and the response to medical therapy is slow. It illustrates also that an elevated alkaline phosphatase level may be observed in association with parenchymal lesions, but very rarely does one obtain an alkaline phosphatase value of more than 10 units without the presence of an obstructive factor. An alkaline phosphatase level which is only slightly or moderately above normal must be evaluated along with the other findings.

3. In many cases of subacute necrosis of the liver or during the acute exacerbation of portal cirrhosis with jaundice (cases 3 and 4), evidence of both acute and chronic parenchymal injury is seen (fig. 6).

CASE 3.—*Subacute Hepatitis (Yellow Atrophy) with Ascites and Jaundice and Subsequent Recovery.*—A 54 year old merchant in Cairo, Egypt, suddenly exhibited jaundice and ascites in May 1947. He came to New York and was

Fig. 5.—The hepatic star in acute hepatitis.

Fig. 6.—The hepatic star in subacute necrosis of the liver or during acute exacerbation of portal cirrhosis with jaundice.

first seen on July 28, 1947. He was icteric and had a few spider angiomas. The liver was at the costal margin. A moderate amount of ascites was present. The stools were normal in color.

Laboratory tests revealed the following results:

	July 1947	November 1947
Icterus index, units	...	7.0
Total bilirubin, mg./100 cc.	3	...
Total cholesterol, mg./100 cc.	152	200
Cephalin cholesterol flocculation	3+	2+
Thymol turbidity, units	...	2.0
Serum alkaline phosphatase, Bodansky units	3.6	11.1
Total serum proteins, Gm./100 cc.	7.0	6.7
Albumin, Gm./100 cc.	2.3	4.1
Globulin, Gm./100 cc.	4.7	2.6
Albumin to globulin ratio	0.49	...

With treatment his condition improved progressively. Ascites was not demonstrable after October 1947, and clinical icterus disappeared soon after. He was discharged from the hospital November 28 as cured. At that time the laboratory findings were as noted in the tabulation.

CASE 4.—*Portal Cirrhosis with Ascites and Terminal Jaundice.*—A 55 year old Puerto Rican merchant had a cholecystectomy in January 1945. At operation the gallbladder was observed to be normal, but the liver was small and nodular. The postoperative course was stormy. Shortly after his recovery from the operation, persistent ascites was noted. When he was first seen in June 1945, the liver and spleen were palpable at the costal margin. There was no jaundice, but he presented the facies typical of hepatic disease, with distended abdomen and spider angiomas.

Laboratory tests showed the following results:

	January 1945	March 1947
Icterus index, units	7.0	25
Total cholesterol, mg./100 cc.	168	125
Cephalin cholesterol flocculation	4+	4+
Thymol turbidity, units	...	0.5
Serum alkaline phosphatase, Bodansky units	5.7	8.3
Total serum proteins, Gm./100 cc.	6.77	6.1
Albumin, Gm./100 cc.	2.17	...
Globulin, Gm./100 cc.	4.6	...
Albumin to globulin ratio	0.47	...

The patient's disease responded slowly to treatment. He gained weight, and progressively longer intervals between paracenteses were observed. In March 1947 he had acute sinusitis followed by bronchopneumonia. Jaundice appeared; the ascites increased, and hepatic coma developed. The patient died during an episode of hematemesis. During the terminal period the laboratory findings were as noted in the tabulation.

Comment.—These two cases are presented jointly because the laboratory findings were similar and, to a certain extent, the clinical signs were the same, in that both patients had ascites and jaundice. However, in case 3 the etiological basis for the clinical picture was subacute hepatitis which responded to medical therapy,

while in case 4 the clinical picture was due to portal cirrhosis with ascites and terminal jaundice. The clinical picture and the laboratory findings must be completely integrated to establish the correct diagnosis and an accurate prognosis.

4. The ordinary patient with portal cirrhosis (cases 5 and 6) is not jaundiced, but injury to the parenchymal cells is present, together with reduction in the serum albumin and ascites (fig. 7).

CASE 5.—*Portal Cirrhosis with Ascites and Without Jaundice.*—A Greek importer, aged 56, with a history of excessive alcohol consumption for years, noted diminishing appetite and weight and the appearance of ascites during a trip to Florida in November 1947. He was under a physician's care the rest of the winter and was first seen on his return to New York in April 1948. At that time he was subicteric with a facies characteristic of hepatic disease. Many spider angiomas

Fig. 7.—The hepatic star in portal cirrhosis.

were present. Ascites was prominent. The liver was palpable 4 fingerbreadths below the costal margin and was hard and nodular. The spleen was just palpable.

Laboratory tests showed the following results:

	April 1948	November 1948
Icterus index, units	8.3	7.2
Total cholesterol, mg./100 cc.	125	...
Cephalin cholesterol flocculation	3+	Normal
Thymol turbidity, units	2.0	1.0
Serum alkaline phosphatase, Bodansky units	3.2	...
Total serum proteins, Gm./100 cc.	5.3	7.2
Albumin, Gm./100 cc.	2.5	3.3
Globulin, Gm./100 cc.	2.8	3.9
Albumin to globulin ratio	0.89	...
Colloidal red test	...	Normal

The patient's condition responded slowly to treatment, and the abdomen had to be tapped at progressively less frequent intervals. However, ascites never completely disappeared.

CASE 6.—*Cirrhosis with Ascites and Without Jaundice.*—A 38 year old white woman, admitted to the University Hospital Nov. 20, 1945, was apparently in good health one year prior to admission, when anorexia, loss of weight and general malaise developed. For several years the patient had been drinking alcohol heavily and gave inadequate attention to her diet. When seen by her family physician she was told she had a serious vitamin deficiency and pellagra. She was given a multivitamin preparation, liver injections and iron. She gained 15 pounds (6.8 Kg.) in weight in three months and felt improved. About three months prior to admission, she noted gradual swelling of the abdomen and, one month later, swelling of the ankles. Paracentesis performed on two occasions yielded 4,500 and 7,000 cc., respectively, of light amber fluid. Three weeks prior to her admission, there was an episode of tarry stools for one week.

Physical examination revealed ascites. The liver and spleen were not palpable. No clinical jaundice was evident.

Laboratory tests showed the following values:

	November 1945	February 1946
Total cholesterol, mg./100 cc.	120	175
Cholesterol esters, mg./100 cc.	55	100
Esters to total cholesterol ratio, %	45	57
Cephalin cholesterol flocculation	3+	2+
Thymol turbidity, units	...	4.0
Total serum proteins, Gm./100 cc.	4.5	5.1
Albumin Gm./100 cc.	2.2	3.1
Globulin, Gm./100 cc.	2.3	2.0
Albumin to globulin ratio	...	1.5
Urinary urobilin, mg./24 hr.	376	...

The patient was placed on a regimen for management of hepatic disease, with definite improvement. She required no further abdominal paracentesis and was discharged in an improved condition on December 12.

She returned to the University Hospital on Jan. 3, 1946 because of progressive enlargement of the abdomen due to accumulation of fluid. At that time an abdominal paracentesis was performed, and 8,500 cc. of pale amber fluid was removed. The patient was discharged on January 7.

Her third admission was on February 2 because of ascites. At that time the laboratory findings were as noted in the tabulation. Abdominal paracentesis yielded 13,500 cc. of clear, straw-colored fluid. The patient was given a course of intravenous liver therapy, with excellent response. She was discharged from the hospital February 18 free of ascites and edema and has remained so since.

5. When complete and uncomplicated obstruction of the common duct is present, as from carcinoma of the head of the pancreas (case 7), the appearance of the star is characteristic (fig. 8).

CASE 7.—*Complete Obstructive Jaundice Due to Carcinoma of Ampulla of Vater.*—A 49 year old automobile salesman began losing weight in April 1945. Painless jaundice developed three weeks before his admission to the University Hospital on May 15, 1945. Severe pruritus was associated with the jaundice.

Physical examination revealed an enlarged, smooth and firm liver, extending to the level of the umbilicus. The spleen was not palpable.

Laboratory studies disclosed an icterus index of 115 units. The total cholesterol measured 210 mg. per 100 cc. The cephalin cholesterol flocculation was normal. Serum alkaline phosphatase measured 9.4 Bodansky units, and the Takata-Ara reaction was negative.

In the duodenal drainage bloody fluid, but no bile, was obtained. The appearance of the gastrointestinal tract on a series of roentgenograms was normal.

At operation a carcinoma of the ampulla of Vater was discovered and removed. The postoperative recovery was uneventful. All test reactions returned to normal. The patient was well until 1949, when he began to lose weight and died from intestinal obstruction due to recurrence of the carcinoma.

Fig. 8.—The hepatic star in complete and uncomplicated obstruction of common bile duct.

Comment.—Study of the duodenal drainage in this case showed biliary obstruction to be complete. The normal cephalin cholesterol flocculation excluded the possibility of associated damage to the parenchymal cells of the liver. This is characteristic of uncomplicated obstructive jaundice. The blood cells in the duodenal drainage permitted a preoperative diagnosis of carcinoma of the ampulla of Vater.

6. When the obstruction of the common duct is due to a stone (case 8), the obstruction is much more likely to be incomplete and intermittent. This will be shown by the study of the duodenal drainage and the intermittent urobilinogenuria, as well as by the clinical signs (fig. 9).

CASE 8.—*Incomplete Obstructive Jaundice Due to Stone in the Common Duct.*—A 58 year old white woman was admitted to the University Hospital on June 8,

1949, complaining of pain in the epigastrium and right upper abdominal quadrant, radiating to the back, and vomiting following meals for the previous six months. Cholecystectomy had been performed in 1929. The patient's stools were not clay colored, and she had only a slight icterus.

Physical examination revealed a soft abdomen with no masses. The temperature was 100.0 F. There was definite epigastric tenderness.

Laboratory tests showed the icterus index to be 25.0 units. Total bilirubin was 2.9 mg. per 100 cc. Total cholesterol was 270 mg., and cholesterol esters were 150 mg. per 100 cc. Thymol turbidity was 3.0 units. Serum alkaline phosphatase measured 8.9 Bodansky units. Serum amylase was 8.0 per cubic centimeter (normal, 1 to 2.5 mg. per cubic centimeter) and serum lipase 1.33 cc. (normal, less than 1 cc.).

Fig. 9.—The hepatic star in incomplete and intermittent obstruction of common bile duct.

A diagnosis of acute pancreatitis was made, and the patient was treated conservatively. Study of the duodenal drainage (after administration of magnesium sulfate) revealed some concentrated bile which contained E. coli. This indicated an obstructive element, possibly a stone in the common duct.

The patient was operated on June 14, at which time a single calculus in a distended common duct was observed. The pancreas was only slightly enlarged. The patient made an uneventful recovery.

Comment.—This case illustrates the association of infection with stone in the common duct and a secondary pancreatitis consequent on regurgitation of infected bile into the pancreas. The increase in the icterus index was slight in this case, as may occur with common duct stone, and the alkaline phosphatase was only slightly elevated because of incomplete obstruction.

7. When obstructive jaundice is complicated by infection and associated cholangitis, as in the presence of stone in the common bile duct or postoperative stricture, or when a carcinomatous obstruction has persisted until secondary atrophic changes occur (case 9), evidences of acute parenchymal injury are added to the picture (fig. 10).

CASE 9.—*Extrahepatic Obstructive Jaundice with Infectious Cholangitis and Obstructive Biliary Cirrhosis.*—A 60 year old white woman was admitted to the University Hospital on May 10, 1945, complaining of jaundice of one month's duration. The past history revealed a similar episode of jaundice lasting one month.

Physical examination disclosed a hard, enlarged liver extending 2 inches (5 cm.) below the costal margin.

Fig. 10.—The hepatic star in obstructive jaundice complicated by infection and cholangitis.

Laboratory studies showed an icterus index of 166 units. The total cholesterol was 195 mg. and the cholesterol esters 50 mg. per 100 cc. The ratio of esters to total cholesterol was 25 per cent. The cephalin cholesterol flocculation was 3+. Serum alkaline phosphatase measured 9.6 Bodansky units. Total serum proteins were 7.1 Gm. per 100 cc., with 2.8 Gm. of albumin and 4.3 Gm. of globulin. Duodenal drainage revealed no concentrated bile. E. coli was present in all specimens.

The diagnosis was obstructive jaundice, infectious cholangitis (E. coli) and early biliary cirrhosis of the liver.

An operation was performed May 25, 1945. The gallbladder was distended and contained several stones. There was a stone in the common duct. The liver was hobnailed in appearance. Cultures of bile taken at operation showed E. coli. A biopsy of the liver revealed biliary cirrhosis and acute pericholangiolitis.

Comment.—The elevated alkaline phosphatase level suggested an obstructive element to the jaundice, while the positive flocculation reaction and reversal of the

albumin to globulin ratio indicated secondary involvement of the liver, a complication which is especially likely in the presence of the infected bile.

8. Acute cholangiolitic hepatitis or early biliary cirrhosis (case 10) shows the characteristics of jaundice and intrahepatic obstruction. There is no obstruction of the common duct. Acute parenchymal injury may be present (fig. 11).

CASE 10.—*Acute Cholangiolitis.*—A white man, aged 37, was admitted to the University Hospital May 6, 1947 because of the onset of jaundice during a therapeutic course of gold preparation injections for arthritis.

Physical examination revealed an enlarged nontender liver 1 fingerbreadth below the costal margin in the midclavicular line.

Fig. 11.—The hepatic star in acute cholangiolitic hepatitis or early biliary cirrhosis.

Laboratory tests showed an icterus index of 125.0 units. The total bilirubin was 10 mg. and total cholesterol 305 mg. per 100 cc. The cephalin cholesterol flocculation was normal. Thymol turbidity was 1.5 units, and serum alkaline phosphatase measured 12.1 Bodansky units.

Bile was obtained on study of the duodenal drainage. However, no concentrated bile was obtained after the administration of olive oil. The culture of the bile was sterile. One week later the bilirubin was 10.6 mg., total cholesterol 320 mg. and the alkaline phosphatase 12.6 Bodansky units, per 100 cc.

The patient was treated with a thorough medical regimen for control of hepatic disease. At no time did any of the reactions to tests for parenchymal cell damage become positive. The icterus index, cholesterol and alkaline phosphatase gradually fell to normal levels.

One year after his discharge from the hospital, the patient was clinically well, with normal laboratory findings.

Comment.—The acute onset of the jaundice resembled that of acute parenchymal hepatitis. However, the normal results on the flocculation tests, associated with

the elevated alkaline phosphatase and blood cholesterol levels indicated some obstruction to bile flow, in this case an intrahepatic obstruction due to cholangiolitis. Complete response to medical regimen ensued.

9. In the late stages of biliary cirrhosis ascites develops (case 11). In this stage all the points of the star are affected except the one indicating obstruction of the extrahepatic bile ducts (fig. 12).

CASE 11.—*Cholangiolitis with Biliary Cirrhosis.*—A 43 year old white woman was first admitted to the University Hospital in January 1949, complaining of jaundice of four years' duration. The icterus was painless and had had an insidious onset. There were intermittent episodes of dark urine and occasional clay-colored stools, but no abdominal pain was present. The history included nothing to suggest acute hepatitis, and there was no record of contact with any hepatotoxic agents or of injections of blood or serum. In 1946 a diagnosis of "cirrhosis" was made, and she was given injections of liver extract and a high vitamin diet without objective improvement in the jaundice. Results of peritoneoscopy and liver biopsy performed at that time were stated to have corroborated the diagnosis of "cirrhosis."

Physical examination revealed an enlarged liver, extending 7 fingerbreadths below the costal margin in the midclavicular line. It was nontender and smooth. The spleen was not significantly enlarged.

Laboratory tests gave the following values:

	January 1949	June 1949
Icterus index, units	75	66.5
Total bilirubin, mg./100 cc.	5.0	5.3
Total cholesterol, mg./100 cc.	875	760
Cholesterol esters	Normal	Normal
Cephalin cholesterol flocculation	1+	2+
Thymol turbidity, units	4.0	1.0
Thymol flocculation	...	Negative
Serum alkaline phosphatase, Bodansky units	20.8	13.9
Total serum proteins, Gm./100 cc.	...	7.9
Albumin, Gm./100 cc.	...	4.8
Globulin, Gm./100 cc.	...	3.1
Albumin to globulin ratio	...	1.5
Urobilinogen units/2 hr.	3.7	1.0
Total galactose excreted on tolerance test, Gm.	2.5	...

Study of the duodenal drainage revealed a 2+ concentration of bile after stimulation with magnesium sulfate and olive oil.

A diagnosis of cholangiolitic biliary cirrhosis was made. The patient was discharged February 1 for a trial period of intensive medical therapy. She was readmitted June 12 with essentially the same complaints, but there was some pruritus as well. The results of physical examination were unchanged.

Surgical exploration was decided on. At operation an enormously enlarged liver was observed. The surface of the liver was moderately irregular, and innumerable dilated bile ducts were seen through the capsule, which gave a mottled appearance to the liver. The spleen was slightly enlarged. There was little evidence of portal hypertension. No stones were present in the gallbladder or

common bile duct. Immediate cholangiography at the operative table showed no evidence of lithiasis in the gallbladder or biliary ducts. A T tube was inserted into the common duct.

Comment.—This patient represents the small group of patients in whom cholangiolitis develops for some unknown reason. The cholangiolitis produces an obstructive type of jaundice. The obstruction, however, is intrahepatic, and the extrahepatic bile ducts are clear. The disease in these patients may progress to cholangiolitic biliary cirrhosis, as illustrated in this case.

Fig. 12.—The hepatic star in late biliary cirrhosis.

SUMMARY

It is obvious that the use of this diagnostic schema rests on the fundamental assumption that the various tests indicate specific types of pathological processes. The distinction among these is not always as sharp as one would wish. The individual clinician may prefer to substitute other tests for those selected here. Such changes need not affect the basic assumptions underlying the use of this schema or the diagnostic value of the interpretations made therefrom.

This method of study does not diminish the value of specific tests, such as the prothrombin time as an index to the control of cholemic bleeding. Many of the tests of hepatic function provide such specific information. The problem of the clinician is to select those functional tests, the results of which will answer the particular question he has in mind. Several methods of study are reported. The hepatic star in my hands has been of great value in teaching, in the study of individual cases and in the differential diagnosis of many of the various medical and surgical types of jaundice.

News and Comment

GENERAL NEWS

Commercial Solvents Award Established for Research in Antibiotics.—The Commercial Solvents Corporation, New York, and the Society of American Bacteriologists announce the establishment of an annual award for outstanding research in the field of antibiotics. The award, $1,000 and a gold medal, will be given to a person or a group of persons working in the Western Hemisphere who contribute to the better understanding of antibiotics. In selecting the winner of the award, particular attention will be given to the basic nature of the research on which the award is made and its contribution to fundamental knowledge about antibiotics.

The Commercial Solvents Award will be administered by the Society of American Bacteriologists, and the recipient will be selected by a committee appointed by the president of the Society. It is expected that the first award will be presented at the annual meeting of the Society of American Bacteriologists in Chicago in May 1951.

PERSONAL NEWS

Dr. Robert W. Berliner.—Dr. Leonard A. Scheele, Surgeon General of the Public Health Service, Federal Security Agency, announced the appointment of Dr. Robert W. Berliner as Chief of the Laboratory and Clinical Section on Kidney and Electrolyte Metabolism of the National Heart Institute. Dr. Berliner was formerly Assistant Professor of Medicine at Columbia University, New York.

Book Reviews

Blood Transfusion. By various authors. Price, $12.50. Pp. 574, with 105 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1949.

What was really the first edition of this book arrived in the United States in 1922 in the form of a monograph 166 pages long, with thirteen illustrations and selling for $3. Its reception varied: It was welcomed coolly in Chicago (*Journal American Medical Association* **79**:762 [Aug. 26] 1922), more warmly in Philadelphia (*American Journal of the Medical Sciences* n.s. **163**:755 [May] 1923), and with open arms in Boston (*Boston Medical and Surgical Journal* **187**:299-300 [Aug. 24] 1922). On the whole it seemed the kind of book which should achieve popularity, for it was well written, with an excellent bibliography, and, as the Boston reviewer pointed out, it was the best and most comprehensive work on the science of blood transfusion that had appeared.

The second edition is longer and much more elaborate. The senior editor (who did most, if not all, of the writing for the first edition) now confesses that he finds himself competent to write only the historical section and that he has enlisted the help of seven collaborators to do the rest so that every phase of blood transfusion shall be described well and with full authority.

The result is a delightful volume. The history of blood transfusion, its indications, its complications, blood grouping, the selection of donors, the technics of transfusion, the storage and preservation of blood and blood derivatives, the use of blood derivatives and blood substitutes and, finally, the organization of a hospital transfusion department are all described clearly, simply and wisely. At the end of each chapter is a carefully selected bibliography. The illustrations are good.

This edition deserves wider and more flattering recognition than its predecessor seemed able to obtain.

Stern's Applied Dietetics. Revised by H. Rosenthal, P. C. Baker and W. A. McVey. Third edition. Price, $5. Pp. 293. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1949.

Stern's "Applied Dietetics" represents a new-fashioned compilation of technical knowledge and practical information concerning normal and therapeutic diets. It is a textbook, the contents of which can be used to explicit advantage in dietary departments responsible for the instruction of patients, student nurses, dietetic interns and public health workers in normal and special diets.

Medical Clinics of North America: Massachusetts General Hospital Number. By various authors. Pp. 287. W. B. Saunders Company, W. Washington Sq., Philadelphia 5, 1949.

These medical clinics are interesting and should be of particular practical value to clinicians, including general practitioners as well as internists. The symposiums include discussions of the management of thyroid disease and some of the mechanisms concerned with the disturbances in physiology resulting therefrom. Other discussions include management of auricular flutter, vascular and cardiovascular hypertensive disease, use of nitrogen mustards in lymphoma, the "dumping syndrome," neurocirculatory asthenia and other important practical clinical problems. This number should be very valuable.

Medical Clinics of North America: Philadelphia Number. By various authors. Pp. 317, with 26 illustrations. W. B. Saunders Company, W. Washington Sq., Philadelphia 5, 1949.

These medical clinics are interesting and should prove to be of value to the practicing physician. Discussions include the management of acute cardiac conditions, massive gastrointestinal hemorrhage, intractable asthma, medical pulmonary emergencies and acute rheumatic disorders. In addition, there is a symposium on nutritional disturbances affecting the heart, diabetic coma, thrombotic phenomena, heart disease and pregnancy and the surgical management of hypertension. These are all timely problems which are important from the practical point of view. This number can be recommended.

Das Menschliche Knochenmark: Seine Anatomie, Physiologie und Pathologie nach Ergebnissen der intravitalen Markpunktion. By Prof. Dr. Med. Karl Rohr. Second edition. Price, 47.50 German marks. Pp. 404, with 143 illustrations. Georg Thieme, Diemershaldenstrasse 47, Stuttgart, Germany, 1949.

The author of this book attempts to cover in a systematic fashion the human bone marrow as it applies to clinical medicine. In order to do this, he divides the book into two main divisions; one dealing with general considerations and one dealing with the special considerations of the problem.

Under general considerations, the author discusses the method of marrow puncture, the embryology and morphology of the marrow and the morphology of the cell. About 45 pages are devoted to the physiology of the bone marrow.

In the latter half of the book the author discusses in great detail the erythrocyte, the thrombocyte, and the leukocyte series with reference to changes in the marrow. In addition to elaborating on the findings in the bone marrow in various infectious diseases, the writer assigns about 40 pages to what he describes as neoplasms of bone marrow.

The book has many excellent illustrations both in color and in black and white. Of particular interest are the many diagrams which the author uses to illustrate in a schematic fashion the various subjects under discussion. Although the book is primarily intended for the persons interested in hematology, it would appeal to any physician who has a reading knowledge of German. There is an adequate bibliography which covers fairly well the pertinent world literature on this subject.

Current Therapy. By Howard F. Conn, M. D. Price, $10. Pp. 736. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1950.

The reviewer wishes that he could give his unqualified approval to this book, which obviously represents an honest attempt to help the physician. A vast amount of material is presented, and the latest methods of treatment are enumerated. In a good many instances, however, various items are simply listed in one-two-three order, sometimes without much comment, and the question is whether this method is a sound one, especially for the physician or student who is not already thoroughly familiar with the given problem. In some sections the directions are much like those for baking a cake or for lubricating an automobile. It seems not without risk to hand to the physician instructions which he may be inclined to follow literally and without question because of their authoritative origin. An example in point is that of some of the severe cardiac arrhythmias. To deal with these properly means watching, judgment and change of pace as the situation develops. Here the cookbook style of directions, for one who is not already expert to his finger tips, may be out and out dangerous.

Thrombosis in Arteriosclerosis of the Lower Extremities. By Edward A. Edwards, M.D., F.A.C.S. Price, $2. Pp. 83, with 41 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1950.

This handsomely printed and illustrated pamphlet is a reprint of one of the American Lecture Series. The subject is covered more or less systematically and while the material is, for the most part, not new it is well presented and readable.

Congenital Anomalies of the Heart and Great Vessels. By various authors. Price, $4.50. Pp. 68, with 80 illustrations. Charles C Thomas, publisher, 301-327 E. Lawrence Ave., Springfield, Ill.

This monograph is an exhibit presented at the meeting of the American Medical Association in 1947. It consists of colored plates of each of several hearts with congenital defects, a photograph of one such heart studied at autopsy, a typical teleoroentgenogram, an electrocardiogram and a brief clinical diagnostic note and a historical note. Only two pages are devoted to each of the sixteen separate defects presented.

It is doubtful that these pages will prove to be of value to anyone. The exhibit most likely was much more valuable when presented and when the authors were present with personal dissertation and discussions.

Autobiography of Dr. Robert Meyer: A Short Abstract of a Long Life. With a Memoir of Dr. Meyer. By Emil Novak. Price, $2.50. Pp. 126. Henry Schuman, Inc., Publisher, 20 E. 70th St., New York 21.

This book ends with the words, "Since December 14, 1945, I have been a proud citizen of the United States of America."

Dr. Meyer gives a delightful account of the circumstances which led to its becoming possible for him thus to end his autobiography. He writes of his early days, of his medical education, of his adventures in general practice, of his researches and, finally, of his climb up the academic ladder which ended, as he puts it, when he was 75 years young and was offered a position at the University of Minnesota.

He tells his story simply and without conceit. It is the kind of a story that any physician will appreciate and that all medical students should read. For Dr. Meyer achieved success by a long and arduous journey—which, after all, is the only way by which any physician can hope to reach his goal.

Röntgen- und Radiumphysik für Mediziner. By Prof. Dr. Richard Glocker. Price, 19.80 German marks. Pp. 275, with 167 illustrations. Georg Thieme, Diemershaldenstrasse 47, Stuttgart, Germany, 1949; distributed by Grune & Stratton, Inc., 381 4th Ave., New York 16.

This, to my knowledge, is the first textbook on radiation physics published in Germany since World War II. As expressed in the title, the book is intended for both the medical student and the practicing physician. This is implied by the German term *Mediziner,* for which, unfortunately (just as for the generic term *Arzt*), the English language still lacks a satisfactory equivalent. Although the volume is concise, the treatment of the subject is comprehensive and covers not only the historical and theoretic aspects but also the practical problems relative to roentgen ray diagnosis and therapy. In addition to special chapters on these practical problems, the book contains chapters on radiation and matter; natural and artificial radioactivity; properties, effects and measurement of radiant energy; primary action on the cell by ionization, and protection against irradiation. A special appendix contains (and explains) the relevant mathematical calculations

and formulas, concluding with a tabulation of important physical and radiologic values. The material is up-to-date, considering the foreign literature extensively and including the latest achievements of neutron radiation, "atomic pile" and such similar subjects. Of particular interest are the critical discussions of the author, who is professor of roentgen ray technic at the Stuttgart Technical Academy. It is also worth while mentioning that, in contradistinction to the old time German textbook with its often complicated and exasperating phraseology and sentence structure, Glocker's book is written in a refreshingly clear and readable style. Anyone able to read German and interested in the subject should greatly benefit by it.

Chemotherapy of Leukemia and Leukosarcoma. By various authors. Price, $4.75. Pp. 54. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1949.

This pamphlet is the outgrowth of an exhibit prepared for a meeting of the American Medical Association and follows the usual manner for presentation of exhibits. Almost every page contains an illustration which is well labeled. There are numerous diagrams, charts, tables and graphs. The data are presented briefly in outline form. There are many practical therapeutic and other clinical facts in these pages. The book should prove to be useful to busy practicing physicians as well as to others who may desire a concise presentation of the subject.

Gemeinsame Erkrankungen aus der inneren Medizin und Chirurgie. By Walther Kanert and Kurt Koelsch. Price, 35 German marks. Pp. 500, with 49 illustrations. Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O (American Zone), 1949.

This volume is the outcome of close collaboration between a German surgeon and a German physician in a military hospital in Poland during the period of 1941 to 1945. In the preface the authors state that while there are numerous and valuable texts on internal medicine and on surgery there is lack of a book whose goal is the presentation of those diseases which incidentally require joint consultation and management on the part of both internists and surgeons. Therefore, the volume is concerned essentially with the problems: In what manner, how long and with what prospect for success is conservative treatment indicated, and what factors indicate a necessity for surgical intervention? Because this implies a rather large order, the authors state that discussions concerning pathogenesis, symptomatology, diagnosis and therapy are presented only to the extent that they are essential to the reader's adequate comprehension.

Much material, some of it presented in conventional manner, is encompassed between the covers of this volume. Trauma and infections are given ample consideration and reflect the experience in this field gained during hostilities. Discussion of diseases of the respiratory, biliary, digestive and urinary tracts bespeaks the authors' familiarity with these systems. On the other hand, the vicissitudes of war, the rapid progress of medical science and the apparent unfamiliarity with current American medical literature of that period are reflected in the pages throughout the volume. For example, there is no reference to protruded intervertebral disk, to cytologic diagnosis of carcinoma in its earlier stages or to the newer approved tests of hepatic and pancreatic function. As regards innovations in treatment, there is no reference, for example, to antibiotics or even to the improved sulfonamides, to hormones in prostatic carcinoma, to the aluminum antacid preparations, to continuous intragastric drip or even to vagotomy in the

surgical treatment of ulcer. Thus an otherwise instructive volume is not up to date in some respects even before it makes its appearance on the dealer's book shelves. However, this is a shortcoming of all books to a greater or lesser degree.

Isotopic Tracers and Nuclear Radiations with Applications to Biology and Medicine. By William E. Siri. Price, $12.50. Pp. 653. McGraw-Hill Book Company, 330 42d St., New York 18, and Aldwych House, Aldwych, London, W. C. 2, 1949.

The medical and biologic sciences are among the chief beneficiaries of the recent practical developments in nuclear physics. A large number of isotopes are now available for qualified research workers and for the treatment of certain diseases. Inevitably, the first scientists to work with stable isotopes and with radioisotopes have been those associated with the development of atomic energy during the war years and those with a comprehensive knowledge of physics. The literature of the medical and biologic applications of nuclear energy is extensive and is widely dispersed in physical journals, technical reports and society proceedings. This is especially true of literature relating to fundamental phenomena and instrumentation. As a consequence of this, the medical or biologic scientist who wishes to perform a certain type of experiment is faced with a difficult task in locating source material and, particularly, in separating the material he seeks from theoretic formulations.

Col. A. P. Gagge, of the Aeromedical Laboratory, Wright Field, Ohio, recognized this problem and supervised the preparation of A. F. Technical Report No. 5669. This report was, in effect, the first draft for "Isotopic Tracers and Nuclear Reactions." Completely revised, the present edition is a useful handbook for anyone who wishes to use isotopes in research.

The first section of the book is a discussion of isotopes and nuclear radiations. The author has done an excellent job in presenting this complicated, technical subject. The general reader, however, should be able to master the basic concepts on which the application of isotopes is based. The second section deals with methods and instruments. It is well written, and, if it accomplishes no other purpose, it will demonstrate the technical problems and instrumental limitations to all who aspire to use isotopes. The reviewer believes that the author has shown good judgment in the presentation of his material. The final section is a review of the medical and biologic applications of isotopes to the end of 1947. This material is presented realistically, and there is less emphasis on the accomplishments than on the unexplored fields. A complete (to the end of 1947) bibliography of work done with each of the isotopes useful in biology is appended. This bibliography is especially valuable because it is arranged by elements.

This volume will certainly be found helpful in every laboratory where isotopes are used or where their use is contemplated. Basically, the work is not a handbook, but it is a synopsis of theory and methods. It can be read with profit by anyone interested in understanding the implications for the medical sciences of atomic physics. The format and the illustrations are excellent, and the style is readable and not at all patronizing.

Resuscitation and Anesthesia for Wounded Men: The Management of Traumatic Shock. By Henry K. Beecher, M.D. Price, $5.50. Pp. 161, with 32 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1949.

As the author states in his preface, the background of this account is wholly military, although there will obviously be many applications in civilian life. Vari-

ous sections deal with the mental and physical state of the wounded man, physical derangements in the wounded found on admission to forward hospitals, treatment of the wounded and anesthetization for the wounded. The book is handsomely printed and beautifully illustrated with photographs taken in the field and seems to the reviewer to represent an up-to-date discussion of the subject from the surgeon's standpoint.

B.C.G. Vaccination in Theory and Practice. By Kenneth Neville Irvine, D.M., B.Ch. (Oxon.), M.R.C.S. (Eng.), L.R.C.P. (Lond.). Foreword by Konrad Birkhaug. Price, $3. Pp. 142. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1949.

This little book deals with a subject about which there still is bitter partisan feeling. The author takes the side in favor of BCG vaccination and presents plenty of evidence for his views. The reviewer feels, however, that no final universal answer can be found, since the indications for BCG vaccination will vary so much with circumstances of time, place and local conditions. Everyone interested in the problem will, however, wish to read this book.

Lehrbuch der röntgenologischen Differentialdiagnostik: Erkrankungen der Brustorgane. By Werner Teschendorf. Second edition. Pp. 779, with 865 illustrations. Georg Thieme Verlag, Diemershaldenstrasse 47, Stuttgart-O, Germany, 1950; sole distributors for the United States and Canada: Grune & Stratton, Inc., 381 4th Ave., New York 6.

This book concerns itself with the differential diagnostic problems of diseases of the organs of the chest as they are observed with the aid of roentgen rays. The author divides the volume into four main parts: (1) differential diagnosis of roentgenograms of the lungs, (2) differential diagnosis of roentgenograms of the heart, (3) differential diagnosis of diseases of the esophagus by roentgen ray and (4) differential diagnosis of the visible changes in the diaphragm by roentgenogram.

It is the reviewer's opinion that this work represents a real contribution to medical literature and will be of great help to any internist who has a reading knowledge of German. The discussions of differential diagnoses are on an anatomic as well as an etiologic basis. In the margins are pertinent notes which refer to the text and permit quick and easy location of the particular subject. The author emphasizes the use of the tomograph and in many instances uses it to demonstrate the superiority of this method over the conventional method. The reviewer believes that the author allocates perhaps too many pages to, and places too much emphasis on the importance of, the kymograph in cardiac diagnosis.

As in any volume of this size, many small errors appear. For example, one wonders why under the heading of "Warning Concerning the Use of Thin Contrast Mediums in Bronchography" the author does not say a word or two about the disturbing and sometimes serious complications which may arise in the preparation of the patient and the instillation of the oil. One might also question the use of hypertension alone as the important differential diagnostic point in the elongation of the thoracic aorta in atherosclerosis as against syphilitic aneurysm.

This work represents the second edition, and it is particularly appropriate to quote from the authors' foreword when, in discussing the difficulty which is not infrequently encountered when one examines a roentgenogram, he uses Goethe's words:

"Was ist das Schwerste von allem? Was dir das Leichteste dünket: Mit den Augen zu seh'n, was vor den Augen dir liegt."
[What is the most difficult thing of all? That which would seem to you to be the easiest: to see with the eyes that which lies before the eyes.]

An Introduction to the Study of Experimental Medicine. By Claude Bernard. Translated by Henry Copley Greene, with an introduction by Lawrence J. Henderson. Price, $3. Pp. 226, with no illustrations. Henry Schuman, Inc., Publisher, 20 E. 70th St., New York 21, 1950.

The reprinting of this classic is pleasing and indicates continued or increasing interest in this excellent book. It should be read by all medical students and young men planning a research career in medicine. Claude Bernard's role in physiology and experimental medicine can never be forgotten. To have an opportunity to profit from the wisdom of such a great man is certainly a privilege. Unfortunately, other great scientists did not make it possible for those who followed them to profit from such advice.

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1. *Reeb, B. B., Rohr, J. R., and Colwell, A. R.: Proc. House Staff Dept. Med., Wesley Memorial Hospital, Chicago, Ill. Feb. 6, 1948.*

2. *Rohr, J. H., and Colwell, A. R., Proc. Amer. Diabetes Assn. 8:37, 1948.*

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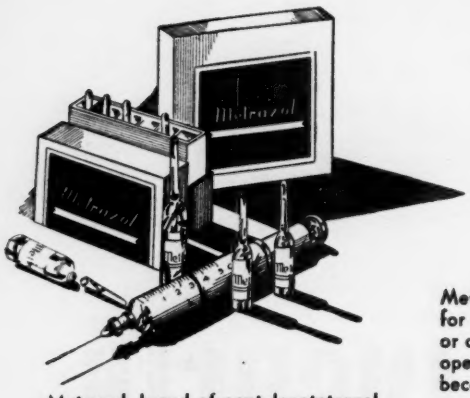

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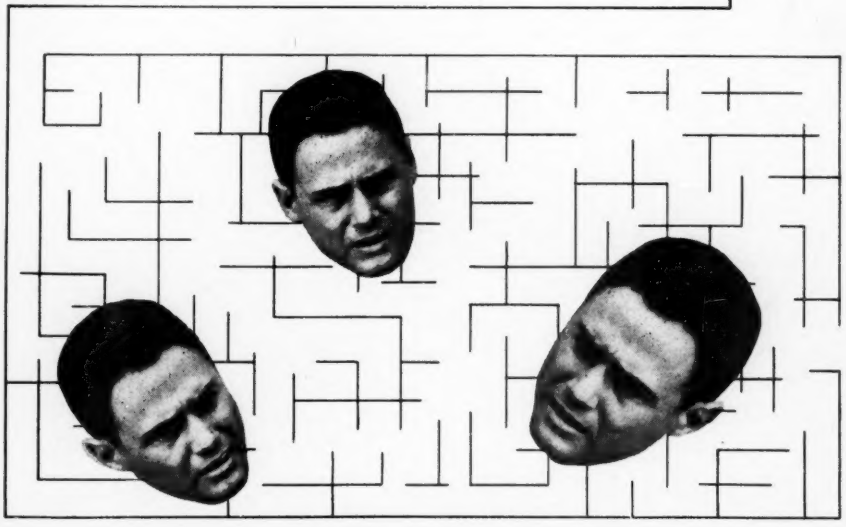
*T.M. Reg. U.S. Pat. Off. for racemic amphetamine sulfate, S.K.F.



Pure Crystalline Vitamin B_{12}

The Only Form Of This Important Vitamin Official In The U. S. P.

PREFERRED BECAUSE

potency, purity, and lack of toxicity of *crystalline* vitamin B_{12} are clearly established.

Potency: Potency of this U.S.P. product is accurately determined by precise weight.

Purity: Pure anti-anemia factor.

Efficacy: Produces, in microgram dosage, maximum hematologic and neurologic effects.

Tolerance: Extremely well tolerated; "no evidence of sensitivity" has been reported.

Toxicity Studies:

In recent pharmacologic investigations, extremely large doses of *crystalline* vitamin B_{12} (1,600 mg./Kg.) caused no toxic reactions in any of the animals treated. In contrast, 3 mg./Kg. of a "*concentrate*" caused fatal reactions in 100 per cent of the animals treated.

Merck—first to isolate and produce vitamin B_{12}—supplies Crystalline Vitamin B_{12} in saline solution under the trademark Cobione.* Your pharmacist stocks Cobione in 1 cc. ampuls containing 15 micrograms of crystalline vitamin B_{12}.

Crystalline Vitamin B_{12}

* *Cobione is the registered trade-mark of Merck & Co., Inc. for its brand of Crystalline Vitamin B_{12}.*

B_{12} COBIONE®

Crystalline Vitamin B_{12} Merck

MERCK & CO., INC.
Manufacturing Chemists
RAHWAY, NEW JERSEY

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